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FROM THE MEDICAL DEPARTMENT OF THE CHILDREN'S HOSPITAL, GOTHENBURG.

The Programme for Premature Infants in Gothenburg, Sweden. The Principles of Treatment and the Results Obtained

by

KURT HOLMDAHL

Since the beginning of 1948, principles in the care of premature infants in Gothenburg, have been applied, which are, to some extent, new. As, since then, considerably improved results have been obtained, the following account has been considered warranted. Since the year 1948 until the end of June 1950, 677 premature infants were cared for, i. e., infants who breathed at birth and weighed 2 500 g or less.

In 1948 and 1949, in the two maternity units in Gothenburg, there was an average of 6 000 living infants born annually, comprising 98 per cent of all infants born in the city. Of these 4.5 per cent were premature. This frequency rate is representative and is relatively low.

The Principles of Treatment

All premature infants born alive in the maternity units are transferred as soon as possible to the two units for premature babies situated within the same hospital. Premature infants born outside the hospitals are, as a rule, not admitted to these units and such cases are not included in this series.

The majority of the mothers attended antenatal clinics during pregnancy. Immediately on admission to the labour ward they receive vitamin K. Consideration of the particular risks involved

if a premature infant is expected influences the method of delivery and drug treatment. These problems are discussed at regular staff-meetings between pediatricians and obstetricians.

The principles of treatment may be conveniently divided into: 1) the care of the infant immediately after birth and the first one to two days of life, and 2) the care after that time. The former period is dominated by measures to maintain respiration and the body temperature, the latter by problems of nourishment.

The measures concerning the regulation of temperature are omitted as they follow the principles generally employed. The measures concerned with respiration are, of course, started by clearing away obstructions of various sorts from the respiratory tract. It should be noted, that if gastro-intestinal administration of oxygen (ÅKERRÉN & FÜRSTENBERG, 1950) is considered, aspiration of the stomach contents, whether these are composed of material swallowed during the delivery or supplied as food, should precede this measure. The administration of oxygen is performed, as a rule, by means of a mask, the smaller infants being placed in incubators. Deeply asphyxiated infants were given oxygen by means of gastro-intestinal administration. All premature infants receive extra administration of oxygen during the first 24 hours of life. In addition to this artificial respiration and stimulants have been used. The latter have, however, been used to a limited extent and in small doses as noteworthy results have seldom been obtained. In many cases good results have been obtained from gastro-intestinal administration of oxygen. Incubators have only been used for the smaller infants and for asphyxiated infants. According to experience from our wards, even very small premature infants can be cared for without difficulty without the additional help of incubators.

To determine the period of time, which should elapse before the first administration of nourishment, the general condition of the infant must be considered. During 1948, food was given at the end of 12 hours. During 1949, the interval was increased up to 36 hours, and at present a longer interval (up to 72 hours) for the smallest infants is employed. The reason for this increasing period of starvation is that severe attacks of cyanosis occasionally

Table 1.

Composition of Human Milk (Macy, 1949). Values are expressed per 150 cubic centimetres of milk. Mean values.

Type of milk. Period of secretion post-partum	Energy calories	Total protein g	Total fat g	Lactose g	Total ash mg	Vitamin A micrograms
Colostrum						
0—5 days.....	100.7	3.4	4.4	—	462	241.5
Transitional milk						
6—10 days.....	110.3	2.4	5.3	9.6	400	132
Mature milk						
≧ 15 days.....	112.1	1.6	6.8	10.7	303	91.5

appear in connection with meal times during the first days. In exceptional cases, during this period of starvation, fluid has been given orally or subcutaneously. We have not, as yet, had much experience of these very long periods of fasting. Cases treated in this way are not included in this report. When the time is considered appropriate for the administration of food undiluted breast milk is supplied from the start. For all infants with a birth weight up to 2 000 g, colostrum is used and for infants over this weight ordinary breast milk. Adequate pasteurised colostrum is available from a breast milk bank so that practically all these infants received colostrum during the time they remained in hospital, i.e., until they attained a weight of 2 500 g.

The reason for choosing colostrum is that its composition in many respects is such that it answers the premature infant's particular requirements to a greater degree than ordinary breast milk.

Table 1 shows average quantities of the most important constituents of breast milk secreted during the different periods of lactation (MACY, 1949). The values have been worked out per 150 ml breast milk as this quantity corresponds to the approximate quantity of fluid, which a premature infant requires in order to satify its fluid requirement per kilo per 24 hours (LEVINE & GORDON, 1942). This quantity should contain ap-

proximately 5—6 g of protein and 100—120 calories, which corresponds to the quantity of protein (LEVINE, 1945) and the amount of energy (GORDON et al., 1940, HESS & LUNDEEN, 1941), which a premature infant is considered to require per kilo per 24 hours. 150 ml of colostrum does not, as a rule, contain this quantity of protein (Table 1) although the quantity of protein is often more than twice that in ordinary breast milk. Therefore, at the beginning, in addition to colostrum two level teaspoonfulls of Aminosol-glucose (MAGNUSSON, 1944) per day were given to the smallest infants, by means of which both the protein and energy requirements are satisfied. As the protein content of colostrum is not infrequently considerably higher (MACY states even up to 6.8 g/100 ml) only this nourishment has been employed since the beginning of 1949. The salt content also is distinctly higher in colostrum (Table 1). Both calcium and phosphorus are found, according to MACY, in larger quantities, which must be considered to be of value for the premature infant. The difference, however, is quite small, and therefore, on a basis of VON SYDOW'S investigations (1946) 50—200 g citric acid milk are given instead of an equivalent quantity of colostrum or breast milk. The relatively low fat content of colostrum should also be of value, as the premature infant as a rule has difficulty in absorbing fat (TIDWELL et al., 1935, GORDON & MCNAMARA, 1941).

The generally accepted principles about the quantities of nourishment to be given are followed, i. e., a low supply of calories in the first week (GORDON, 1943). After about 12 days the quantity of nourishment is, as a rule, such that it answers to the requirements previously mentioned.

For the smallest infants it is important at the start that the quantity per meal does not excede 4—5 g. Vomiting is often a sign that the amount of feed is too large and should indicate a reduction.

From the age of 4 weeks all infants received iron in the form of "Guttafer" (FERROSAN, Malmö, Sweden) 15 drops twice daily, which corresponds to 50 mg ferrous iron. This has, in practically all the cases, been well tolerated. In accordance with previous experience from other quarters (ABT & NAGEL, 1932, MERRITT &

DAVIDSON, 1934 et al.) in the majority of cases the so called early anemia has not been prevented but an anemia has appeared, which in its degree of severity has most often been inversely proportional to the birth weight. Despite the fact that in some cases the hemoglobin value fell to 25—30 per cent of normal, blood transfusion has not been used in a single case in this series.

Administration of vitamin D has been commenced at the age of one week in small doses increasing to a daily dose for the smaller premature infants of 8 000—10 000 units D_2 or D_3 at the age of approximately three weeks. This corresponds approximately to the dosage recommended by PARK (1940) and MALMBERG (1941—1944). The vitamin B complex and vitamin C have been administered commencing in the first week of life in the usual doses. Vitamin K has been administered either orally or by injection during the first week of life. The high vitamin A content of colostrum (Table 1) has been considered sufficient for the infants' requirements.

The prevention of infection has to a great extent been facilitated by the fact that no premature infants other than those born in the hospital were admitted. Great stress has been laid on the fact that the premature infants who are considered particularly exposed to risk of infection, are treated immediately with penicillin. This holds good for infants born to mothers with signs of intra-uterine infection or infants, who are asphyxiated or show signs of lesions in connection with the delivery.

One of the main principles in the care has been to reduce all handling of the infants to a minimum and therefore all clinical and laboratory investigations have been reduced as much as possible.

Results of Care

In order to judge the results of different methods of care one has, to compare the morbidity and mortality rates in different series of premature infants. The method often relied upon, to assess different methods of feeding on a basis of increase in weight, is less valuable as long as the optimal weight curve is unknown.

In the following account only the figures for mortality will

be reviewed for two reasons: Firstly, it is very difficult to compare the figures for morbidity from different hospitals, as the figures of the frequency depend upon the intensity of the diagnosis and the definitions of the different conditions of illness to a great extent, and secondly, it is to be presumed that the morbidity rate and the mortality rate run more or less parallel and, thus, the mortality rate gives a good impression also of the morbidity rate.

The best method of obtaining completely comparable series would be, of course, that every alternate infant was treated according to a certain method and the intervening infants left untreated. Such a procedure was, unfortunately, not adopted in our cases. Another method is to compare results with previous results from the same hospital. Here it must be pointed out, that a considerable reduction in the mortality has occurred since the present regime was introduced to the hospital. These figures have previously been reviewed by ÅKERRÉN (1949). The third method is to compare the mortality rate in premature infants from different hospitals. Such comparison is, however, often associated with great difficulties depending primarily upon differences in the basic material. The following demands must be satisfied in order that the different series are suitable for comparison: 1) The maternal material should be representative or similar. 2) The concept live born premature infants must be clearly defined and similar. 3) All live born premature infants in the maternal population in question should be included. 4) The distribution in weight groups should be given and the mortality compared in the different weight groups. 5) The observation time should be stated and should preferably be similar in the different series.

In table 2 some investigations are included, where these demands, taken by and large, can be considered to be satisfied. The choice of series for comparison depends in addition on the desirability of obtaining a representative Swedish material and to include both the best English and American series of major extent, which are encountered in the literature. The Birmingham investigation (England) was carried out by CROSSE (1949),

Table 2.

Distribution of premature live births by weight groups and neonatal mortality rate.

Birth-weight in grams	Birmingham 1931—1946			Kristianstad 1940—1947			Denver, Colorado 1946—1949			Gothenburg 1948—1950		
	Number of cases	%	Mortality within 4 weeks %	Number of cases	%	Mortality within one year %	Number of cases	%	Mortality within 4 weeks %	Number of cases	%	Mortality within 4 weeks %
≦ 1 000	21	1.8	96.6	3	0.6	100	34	7.0	91.2	40	5.9	97.5
1 010—1 500	94	8.2	62.7	69	12.7	72.5	57	11.9	38.6	74	10.9	60.8
1 510—2 000	238	20.9	20.1	139	25.6	28.8	101	21.0	14.9	147	21.7	8.8
2 010—2 500	788	69.1	3.8	333	61.3	8.4	288	60.0	2.8	416	61.4	5.8
Total	1 141	100	13.4	544	100	22.2	480	100	15.8	677	100	18.1
1 010—2 500	1 120	—	12.2	541	—	21.8	416	—	10.1	637	—	12.9
1 010—2 000	332	—	32.2	208	—	43.3	158	—	23.3	221	—	26.2

the investigation in Kristianstad (Sweden) by SELANDER (1949), and the investigation in Denver (U.S.A.) by TAYLOR, PHALEN & DYER (1949). In Birmingham and Kristianstad the infants were fed on breast milk, while a cow's milk mixture for the smallest premature infants was used in the American material. It is apparent that the lowest figure (Table 2), 13.4 per cent, for all weight classes together was attained in Birmingham. This favourable figure depends, however, partly on the fact that the distribution of the premature infants in the different weight groups in this material is different from at least two of the other series. Thus, in the lowest weight group only 1—2 per cent of the infants are represented just as in the material from Kristianstad. A comparison between the mortality rate for premature infants between 1 010—2 500 g therefore does more justice to the different materials. The mortality in the series from Birmingham, Denver and Gothenburg is found to lie between 10.1 and 12.9 per cent; the best result being from Denver. In Kristianstad there was a mortality within 4 weeks of 20 per cent for all premature infants. The figures for the weight group over 1 000 is not stated. In the group 1 010—1 500 g a very low mortality is found in the American series, while in the weight group 1 510—2 000 g the figure in the Gothenburg material is the best. For the group between 1 010—2 000 g it appears that the figures in the Gothenburg and Denver material are the best.

Judging from the figures from Denver, it thus seems that very good results can be reached by artificial feeding of premature infants. The results in the lowest weight groups under 1 500 g are particularly good. The difference between the Denver and Gothenburg material is in the class 1 010—1 500 g 2.2 ± 8.59 % (i. e., 2.6 times the mean error). Whether this probable difference is due to the different methods of feeding or to other factors, is naturally impossible to judge. The fact that in the series from Denver approximately 15 per cent were negroes possibly plays some part. On the other hand, the figure from Gothenburg between 1 510—2 000 g is, as already mentioned, the lowest. The differense between the series mentioned amounts here to 6.1 ± 4.24 per cent. The difference between the Birmingham and the Gothen-

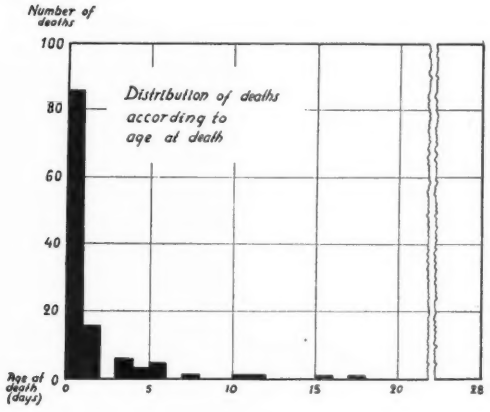

Fig. 1.

burg material in the same weight group is 11.3 $\pm$ 3.51 per cent. Thus it seems that feeding according to the method applied here, in the weight group 1 510—2 000 g, gives very favourable figures.

Discussion

Finally, the question arises: which changes in the principles of treatment hitherto employed can be considered warranted on a basis of this comparison between the results from different hospitals? Before an answer is given, it is of interest to study fig. 1, which shows the distribution of the deaths in the Gothenburg series according to the age in days. The majority of the deaths, 84.1 per cent, occurred during the first two days of live and only 15.9 per cent after this time and until the age of 4 weeks. The mortality risk after the elapse of two days (Table 3) was distinctly higher in the Kristianstad and Birmingham series (the difference between the Gothenburg and Birmingham series only, however, 2.5 $\pm$ 1.03 per cent). In the American series, unfortunately, no corresponding figures are stated but Dunham (1948) states, on a basis of the official reports from U.S.A. 1944, that approximately 57.1 per cent died during the first 24 hours of life.

Table 3.

Percentage distribution of neonatal deaths according to age at death and the mortality risk after the elapse of 48 hours.

	Birmingham	Kristianstad	Gothenburg
Number of deaths	157	121	121
Under 24 hours	50.6 %	44.0 %	70.8 %
24—48 hours	11.0 %	14.7 %	13.3 %
Under 48 hours	61.6 %	58.7 %	84.3 %
48 hours to 4 weeks	38.4 %	41.3 %	15.7 %
No. of infants at commencement of period of observation	1 141	544	677
Surviving after 48 hours	1 044	473	576
Number of deaths 48 hours to 4 weeks	60	50	19
Risk of death after 2 days	5.7 %	10.6 %	3.3 %

It therefore seems that the mortality risk in the series described here is relatively low after the elapse of 48 hours. This seems to be in favour of the fact that the measures for prevention of infection and for the administration of nourishment employed have been to a great extent satisfactory. Considering that the principles of feeding, apart from the feeding with colostrum in the Gothenburg series, were rather similar in these three reports from Kristianstad, Birmingham and Gothenburg, could it not then be asked if colostrum has not played some part? The difference established, which is present between the mortality for premature infants in the weight group between 1 510—2 000 g, is of particular interest because just in this weight group there is reason to suppose that an improved form of nourishment exercises an influence on the mortality rate. No definite opinion can however be given on account of the differences, which are often difficult to define, which are found between material from different quarters and from different periods of time.

An investigation of the causes of death after the elapse of 48 hours in the Gothenburg series showed that brain injuries,

debility and deformities were the cause of 13 deaths and that infection was the cause in 6 cases. As a rule, aspiration has been concerned in these cases. Disturbances of nutrition have thus not occurred. From this it will also appear, that the methods for prevention of infection and the administration of nourishment are rather satisfactory and that by means of improving these methods, no major reduction of the mortality rate in premature infants can be attained. The conclusion must be that it is primarily the mortality during the first 48 hours against which efforts must be directed. An increased consideration for the premature infant from the obstetrician's point of view and an additionally prolonged fasting period before the commencement of administration of nourishment are those measures, which now appear to be the most desirable.

Summary

Since the commencement of the year 1948, distinctly improved results have been achieved in the care of the premature infants in Gothenburg. An account is given of some new principles of treatment. The value of oxygen administration, particularly gastrointestinal administration, and the theoretical (Table 1) and practical advantages of human colostrum as a nourishing medium for the premature infants are stressed.

In order to judge the results of care, the mortality in three other series of premature infants is compared with the author's own series (Table 2). An analysis of the mortality in the different weight groups shows that a very favourable figure was reached in the Gothenburg series in the weight group 1 510—2 000 g. For the weight groups 1 010—1 500 and 2 010—2 500 g, the mortality was lowest in the series from Denver.

From the distribution of the deaths with a considerable peak immediately after birth (Fig. 1) and from the nature of the causes of death it appears that disturbances of nutrition and infections were seldom the cause of death.

A more substantial reduction of the mortality for premature infants in Gothenburg is only possible by reducing the mortality in the first 48 hours. An intensified cooperation between pediatricians and obstetricians is necessary.

Le programme concernant les enfants prématurés à Gothembourg (Suède). — Principes thérapeutiques & résultats obtenus.

Depuis le commencement de l'année 1948, des résultats nettement améliorés ont été apportés en ce qui concerne la protection des enfants prématurés à Gothembourg. Un compte rendu est donné des nouveaux principes de traitement. L'auteur insiste particulièrement, d'une part sur la valeur de l'administration de l'oxygène, d'autre part sur l'alimentation, et, à ce sujet, il montre les avantages théoriques (tableau 1) et pratiques du colostrum humain comme facteur alimentaire chez les enfants prématurés. Afin de juger des résultats obtenus, il compare la mortalité dans trois autres séries d'enfants prématurés à la sienne propre (tableau 2). Lorsqu'on analyse la mortalité pour des poids différents, il ressort de cette comparaison que l'avantage reste à Gothembourg pour des poids compris entre: 1 510 & 2 000 grs. Parcontre, pour ceux compris entre 1 010 & 1 500 d'une part, et, 2 010 & 2 500 d'autre part la mortalité se montre plus basse dans les séries de Denver. Pour expliquer la répartition des cas mortels dont la courbe montre une ascension considérable immédiatement après la naissance (fig. 1) il apparait, de toute évidence, que les facteurs principaux sont rarement les troubles de nutrition & les infections. Une réduction plus importante de la mortalité des enfants prématurés à Gothembourg n'est possible que grâce à la régression de la mortalité dans les premières 48 heures. Il apparait donc absolument nécessaire qu'il y ait une étroite coopération entre les pédiatres et les accoucheurs.

Das Behandlungsprogramm für prämature Kinder in Gotenburg, Schweden. Die Grundsätze der Behandlung und die erzielten Resultate.

Seit Beginn des Jahres 1948 sind deutlich bessere Resultate bei der Pflege der frühgeborenen Kinder in Gothenburg erzielt worden. Der Verfasser berichtet über die teilweise neuen Behandlungsgrundsätze. Er unterstreicht den Wert von Sauerstoffzufuhr, insbesondere gastrointestinaler, sowie die theoretischen (Tab. 1) und praktischen Vorzüge von humanem Colostrum als Nahrungsmittel für vorzeitig geborene Kinder. Um die Behandlungsresultate beurteilen zu können, vergleicht er die Mortalität in drei anderen Serien prämaturer Kinder mit seiner eigenen Serie (Tab. 2). Eine Analyse der Sterblichkeit in den verschiedenen Gewichtsgruppen ergibt, dass in der Gotenburger Serie eine sehr günstige Ziffer in der Gewichtsgruppe 1 510—2 000 g erreicht wurde. Bei den Gewichtsgruppen 1 010—1 500 und 2 010—2500 g war die Mortalität in der Serie von Denver am niedrigsten. Aus der Verteilung der Todesfälle

mit einem bedeutenden Gipfel unmittelbar nach der Geburt (Fig.1) und aus der Natur der Todesursachen geht hervor, dass Ernährungsstörungen und Infektionen selten die Ursache des Todes waren. Eine stärkere Herabdrückung der Mortalität frühgeborener Kinder in Gotenburg ist nur möglich durch Verminderung der Sterblichkeit in den ersten 48 Stunden. Hierzu ist eine enge Zusammenarbeit zwischen Pädiatrikern und Obstetrikern erforderlich.

Programa para los niños prematuros en Gotemburgo. (Suecia). Principios en que se basa el tratamiento y resultados obtenidos.

Desde el comienzo del año 1948 se han obtenido resultados positivos y evidentes en el cuidado de los prematuros en Gotemburgo. Se hace una relación de los nuevos principios en que se basa el tratamiento y se resalta el valor de la administración de oxígeno particularmente por via gastrointestinal y las ventajas teóricas (tabla 1) y prácticas que representa el calostro como medio alimenticio para el niño prematuro.

En orden a juzgar los resultados de esta protección se compara la mortalidad de otras 3 series de prematuros con las series propias del autor (tabla 2). Un análisis crítico de la mortalidad en los diferentes grupos según el peso muestra unos resultados favorables para las series de Gotemburgo en les pesos comprendidos entre 1,510—2,000 gr. Para los grupos de peso entre 1,010—1,500 y 2,010—2,500 la mortalidad era más baja en las series de Denver. Del estudio de la distribución de los casos mortales con una considerable cumbre inmediatamente tras el nacimiento (figura 1) y de la naturaleza de las causus de defunción parece evidente que los trastornos de la nutrición y las infecciones raramente eran la causa de la muerte. Una mayor reducción substancial de la mortalidad en los niños prematuros de Gotemburgo es solo posible reduciendo la mortalidad en las primeras 48 horas de la vida y para ello es necesaria una muy estrecha colaboración entre los pediatras y tocólogos.

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Congenital Toxoplasmosis[1]

by

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Toxoplasmosis seems to be indigenous in a large number of countries. Recent investigations have demonstrated, also, that in many parts of the world the incidence of infection is much higher than has previously been supposed. Further, the injurious effect on the organism is in many cases severe. From the medical point of view, the disease is therefore of considerable significance.

As far as can be judged at present, the disease is of greatest clinical significance when it attacks pregnant women. In these cases, the infection is often communicated to the fetus, which seems to be extremely susceptible. Our knowledge regarding the conditions governing this transference of infection to the fetus is as yet very limited. One of the conditions seems to be that the mother should be infected during the course of the pregnancy. If a woman already displaying clinical and serologic signs of toxoplasmosis becomes pregnant, the risks of the fetus becoming infected seem to be comparatively small. It is possible, however, in exceptional cases, that infection of the fetus with toxoplasma may occur in two successive pregnancies.

If infection occurs at an early stage in pregnancy there seems to be a risk that fetal death and abortion will result. If it occurs towards the end, the process seems to develop more slowly, the infant is delivered prematurely, or at term, but with signs of more or less severe affection. The infection produces fetal, or

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congenital, encephalomyelitis, the eyes, and especially the retina, becoming affected. The result is a characteristic syndrome: congenital toxoplasmosis. The first definitely established case was described in 1939[1]. The main signs and symptoms in this form of toxoplasmosis, which has been discussed in a number of publications from this country (2—18), are as follows: Hydrocephalus with yellowish cerebrospinal fluid having a high protein content, cerebral calcifications, chorioretinitis, convulsions and mental backwardness.

Internal hydrocephalus is often present. According to WALSH (19), it is found in 80 per cent of all cases. The cause is obstruction of the aquaeductus cerebri or the foramen interventriculare, or of both, due to an inflammatory exudate or necrotic masses. This hydrocephalus is sometimes severe, reducing the brain to a thin layer of cerebral tissue. In some cases it causes severe complications during delivery. In place of hydrocephalus, microcephaly is sometimes found, due, in all probability, to advanced antenatal necrosis of the brain. In typical cases, the ventricular fluid has a high concentration of protein; values as high as 1 390 (20) and 2 600 mg per cent (21), for instance, have been encountered. It has a characteristic yellow colour and contains numerous toxoplasma. It is important to bear in mind, when making an examination, that the communication between the ventricles and the lumbar canal, as mentioned before, may be blocked. If this is so, the lumbar fluid might be almost normal and without toxoplasma, while fluid from the ventricles will contain a large number of parasites.

Cerebral calcifications, according to BAMATTER (22), occur in roughly one-half of the cases previously described, and are demonstrable in ordinary roentgenograms of the head. These calcified toxoplasma foci are often encountered lateral to the ventricular system, but they may also occur in the choroid plexuses, the basal ganglia and the thalamus. In the X-ray picture, they often appear as dots scattered about in the hemispheres. This sign is not specific, but when observed in conjunction with other signs and symptoms it is of great diagnostic value.

The *chorioretinitis* not infrequently is bilateral, and occurs in the macular region in most instances. After a period of progression of varying duration the lesions heal up leaving atrophy and pigmentation. According to some investigators, these lesions have a specific appearance. In the opinion of GRANSTRÖM (12) and many other ophthalmologists, on the other hand, they are indistinguishable from other healed lesions, as far as their appearance is concerned. In severe cases, the entire uvea is affected, the condition resembling a chronic uveitis. A secondary cataract then

arises in many instances, and some of these patients subsequently go blind. Microphthalmus is also present in some cases, indicating that the infection attacked the eye at a relatively early stage of development (6 months).

Generalized or local convulsions often occur (in 60—70 per cent (19)), in some cases, however, not until a later stage of the disease. Generalized fits are often followed by transient spinal and bulbar symptoms, as paresis of the limbs, throat, or respiration.

Mental deficiency is observed in the majority of the severe cases. In some patients the mental development is only slightly retarded, in others almost all mental faculties are lacking.

In addition a number of uncharacteristic and less common signs and symptoms may be encountered in congenital toxoplasmosis. This is only natural, considering that changes due to toxoplasma can occur in almost every organ of the body.

A few cases display inability to regulate the body temperature, for instance, and fluctuations amounting to several degrees can occur (23, 16, 17). In some instances, severe hypoprothrombinemia (24, 16) is observed, and in a few isolated cases hypoprothrombinemic hermorrhages (2) occur in association with the latter condition. The blood picture is characterized by eosinophilia of a high degree in some patients. Enlargement of the liver and spleen is often encountered; according to ZUELZER (23) it occurs in 75 per cent of the cases. Jaundice has been noted in a few cases.

Deformities have also been reported in connection with prenatal toxoplasma infection (25).

All these cardinal symptoms may be present in typical cases of congenital toxoplasmosis. One or several of them may be absent in some patients. Unilateral chorioretinitis with a subsequent reduction in visual acuity, and strabismus, may, for instance, be the only demonstrable sign of congenital toxoplasmosis (12, 13, 26). In GRANSTRÖM's strabismus material, comprising about 1 000 cases, there were 10 patients in whom the squint was caused by a macular focus in one eye. Six of these 10 children, as well as their mothers, were serologically positive. This high proportion of positive reactions in the age-class in question here, as well as the fact that the mothers also showed a positive reaction, suggests that congenital toxoplasmosis must have been the cause of the macular lesions in these six cases.

Not even in typical cases of congenital toxoplasmosis should a diagnosis be made on the basis of the clinical findings alone; the final decision should rest on the laboratory data. There is, as a matter of fact, a form of congenital encephaloretinopathy which resembles congenital toxoplasmosis but has a different etiology, at present unknown (27).

The prognosis varies considerably from case to case in congenital toxoplasmosis. The infant is sometimes stillborn, or may die shortly after birth. Those infants that survive not infrequently have serious defects due to deleterious injuries in the central nervous system and eyes. In other cases, as was mentioned before, the only demonstrable symptom may be strabismus, for instance.

We do not know whether fetal toxoplasma infection can run a subclinical course. No definite information on this point seems to have been reported as yet. We had an opportunity of following up several infants who had a high antibody content in the umbilical cord blood at birth but no other clinical signs or symptoms (28). In all these infants the antibodies disappeared from the blood after a relatively short period, indicating a transplacental transference of antibodies from the mother rather than an antenatal infection of the child. Transplacental transfer of antibodies have been reported by other workers also (29, 30).

The present paper describes a number of cases interpreted as prenatal toxoplasmosis, the diagnosis having been based primarily on laboratory findings. The direct observations were made during the period 1948—1950, and at the time of the early examinations the diagnostic methods were still rather unsatisfactory. For the most part, however, blood samples obtained at various examinations were preserved in a frozen state, and as better methods became available they were re-examined. The figures given in this paper are based on titrations carried out in accordance with a standardized technique and are thus quite comparable with one another. This is of some significance as patients were studied over a long period and numerous samples were taken.

The serologic methods mostly used in the investigation were as follows:

(a) The dye test worked out by SABIN and FELDMAN. If the controls are carefully selected, this technique has proved to give satisfactorily reproducible results. It is of the utmost importance, however, that the "accessory factor" serum used is continuously controlled and that the amount of accessory factor in the tests is adequate. We have found, in examinations on several hundred blood donors, that less than 10 per cent of sera from healthy adults are suitable for use as accessory factor. It is not at all unlikely that the inconsistent results obtained at various laboratories when using this method may have some connection with difficulties in this respect.

(b) The complement fixation reaction. Extracts from infected chorioallantoic membranes were used as antigen, after centrifugation at 13.000 r.p.m. in a Sorvall angle centrifuge.

The results of the two methods show fairly good agreement. The correlation seems to be especially good in repeated examinations on one and the same patient. On the other hand, the ratio of the two titer values seems to vary from case to case; this possibly suggests that the antibodies responsible for the two reactions are not absolutely identical.

Toxoplasmin used for intracutaneous tests was identical with the egg antigen employed in complement fixation reactions. It was standardized by titration of complement fixing capacity as well as by testing on persons whose type of reaction was known from previous examinations. In general, a concentration of 0.4 per cent was used.

Case reports

Case 1. A female infant, born Oct. 4th, 1949, 3—4 weeks before term. Weight at birth 2 800 g.

Mother aged 22, father 26, both healthy. The father was a farmer, and both he and the mother had always lived in the country in close contact with animals of various kinds. The mother was a primipara and had had no abortions. Both the present pregnancy and the delivery had been normal in every respect.

The infant was slightly pale and had moderately severe anemia at birth. Examinations carried out on account of this condition revealed that both mother and infant were Rh positive and that the Wassermann reaction was negative in the mother's blood. At the age of 7 days the infant was transferred to a local children's hospital. It now had severe conjunctivitis in addition to anemia but no other symptoms of interest. A few days later, however, the fontanelle showed signs of increased tension. The circumference of the head, which had been 32 cm at birth, had increased to 37 cm by the 14th day of life and at 1 month it was 40 cm. By this time, the frontal fontanelle was noticeably bulged and tense, and rupture of the sutures had obviously taken place. A lumbar puncture was carried out but was not very satisfactory owing to bleeding from the wound; however, 30 leukocytes to 2 000 red blood cells were counted. The Wassermann reaction in cerebrospinal fluid was negative. The child's general condition was good. It sucked well at the breast and gained normally in weight.

At the age of 5 weeks the child was sent to a neurosurgical clinic in

Stockholm because of the hydrocephalus. X-ray examination of the head performed at the clinic showed a normal skull. Symmetrically placed near the anterior portion of the fissure of Sylvius, on both sides, there was irregular, streaky calcification over an area the size of a hen's egg. There were no other signs of abnormality. A ventriculographic examination was carried out on the same occasion and revealed pronounced symmetrical distention of the lateral ventricles and the third ventricle. A layer of connective tissue slightly more than one centimeter thick was all that was left between the skull and the lateral ventricles. The 2 mm segment situated nearest to the third ventricle was the only visible part of the aqueduct. The third ventricle was situated in the midline. The fourth ventricle was also filled with air at this examination; it was seen to be slightly dilated, and was situated further towards the front and higher up than is normally the case, but was not deformed. Neither the cisterna magna nor the subarachnoid space in the neck were air-filled. Thus, a relative stenosis of the aqueduct and an obstruction in the passage between the ventricular system and the cisternae was present. This condition was not studied in more detail (pneumography after the injection of oxygen into the cisternae at the back of the cranium).

The result of a blood test carried out on the mother 30 days after delivery was now received. As this showed a positive dye test (1: 2 000) both mother and infant were moved to the Sachs' Hospital for Children for continued observation. When admitted to this hospital the infant was 46 days old. Physical examination revealed an afebrile, pale, typically hydrocephalic child with a head having a circumference of 42 cm and a suture of 1—1 1/2 cm. The frontal fontanelle bulged slightly, and was 7 × 8 cm wide. Examination of the internal organs yielded nothing of interest. A detailed examination of the eyes (Dr. GRANSTRÖM) showed no outward signs of abnormality. No irritation of the iris and no beam. The irides and pupils were normal. The media were clear. In the retina immediately above and slightly temporal to the left papilla, there was an oblong lesion slightly more than twice the size of a papilla, showing the features of a chorioretinitis. This appeared to be healed, and was for the most part atrophied and slightly pigmented. Except for mild spasticity no features of interest were obtained from the nervous system.

Laboratory data. On admission, the Hb. was 57 per cent. Leukocytes 16 500— staff cells 3 per cent, segmented neutrophils 16 per cent, eosinophils 5 per cent, monocytes 2 per cent, lymphocytes 74 per cent. Two days later, after a bloodtransfusion, Hb 82 per cent, erythrocytes 4.2 million. Ventricular puncture was carried out on the 51st day of life and yielded a bright yellow fluid. 30 ml was removed. Nonne + + +, Pandy + + +. Total protein concentration 240 mg /100 cc, cerebrospinal fluid sugar 52 mg/100 cc, chlorides 425 mg/100 cc. In smears for direct observation, toxoplasma-like bodies were observed. Inoculation of mice

Date	Dye test		Complement fixation reaction		Toxoplasmin skin test		Other data
	Mother	Infant	Mother	Infant	Mother	Infant	
4.10.49	—	—	—	—	—	—	Partus
3.11.49	1: 2 000	—	—	—	—	—	—
19.11.49	—	—	—	—	Pos.	Neg.	—
22.11.49	1: 1 000	1: 500	—	1: 30	—	—	—
28.11.49	—	—	—	—	—	Neg.	—
30.11.49	1: 1 000	1: 500	1: 60	—	—	—	—
16. 1.50	1: 500	1: 500	—	—	—	—	—

and guinea-pigs with the fluid produced typical toxoplasmosis in the inoculated animals and a strain could be established in passages.

Case 2. A female infant, born June 12th, 1947, at term. Weight at birth 3 020 g.

Mother and father both aged 25. The father had always enjoyed good health. The mother had had polyarthritis at the age of 18 but had subsequently had no ilnesses except for a few acute respiratory tract infections. During the first month of her pregnancy she had worked at a butcher's shop, where she was chiefly engaged in cleaning rabbits and poultry. The parents lived at Visby.

At the age of 2, on June 21st, 1949, the infant suddenly became ill towards the evening. She had generalized convulsions, foamed at the mouth and became cyanotic. The attack passed over within a few minutes. The patient was sent to the children's clinic at the Visby Hospital because of this attack. Examination on admission yielded the following information. Weight 12.4 kg. General condition unaffected. There was no cyanosis but the face was fairly red. There was a number of small non-tender swollen glands at the front of the neck. Temperature 39.6° C. Throat slightly inflamed. The internal organs appeared to be normal. Nothing of interest obtained from the nervous system. There was alternating convergent strabismus and nystagmus; the infant had had these two symptoms since its birth. The temperature dropped to normal after only one day at hospital. The infant was kept at the clinic for observation for three weeks. During this time the temperature remained normal. The erythrocyte sedimentation rate varied between 6 and 10 mm in 1 hour. The leukocyte count was 4 500. There were no further convulsions. Lumbar puncture yielded a clear, colorless fluid. The Pandy and Nonne tests were negative. There were 5 mononuclears, 1 polynuclear and 36 erythrocytes to a cubic millimeter. Wassermann reaction negative. Examination of the eyes by an ophthalmologist gave the same result as an examination carried out under an anesthetic on

April 2nd, 1949: Microphthalmus and congenital convergent strabismus of the right eye. Papillae pale. Changes in the form of chorioidal atrophy and pigmentation in both retinae. Visual acuity greatly reduced in the left eye also, this being obvious from the infant's violent "searching" nystagmus.

Because of this finding of old chorioiditic lesions close to the papillae on both sides, toxoplasmosis was suspected. An x-ray examination of the head carried out with this condition in mind yielded further evidence in favour of this diagnosis. There were numerous calcified areas in the chorioid plexus as well as in the cerebrum in general. The patient also displayed marked mental backwardness. The degree of backwardness was not established with certainty, however. Serologic tests were carried out, and the results are shown in the table. The clinical signs and symptoms in conjunction with the results of the serologic tests point strongly to congenital toxoplasmosis in this case.

The infant was discharged and the parents advised to return to the hospital if any further attacks of cramp occurred. On Dec. 24th, 1949 a new attack suddenly set in, with foaming at the mouth, tonic cramp of the trunk, irregularity of the respiration, and a rapid pulse. The patient was hospitalized for four days. As on the previous occasion, this attack also had set in in connection with an acute infection, this time with an initial temperature of 38°.4 C.

On Jan. 28th, 1950, the patient was re-examined at the outpatient department. She had had no further fits in the meantime. No other features of interest were observed.

Months after delivery	Dye test		Complement fixation reaction	
	Mother	Infant	Mother	Infant
25	1:500	1:500	1:28	1:64
31	1:50	1:50	—	1:128

Case 3. The mother, aged 27, was a primipara and showed no signs of illness. She was examined at regular intervals during her pregnancy, which ran a normal course.

The infant, a male, born 2—3 weeks before term, weighed 3 000 g at birth. He was well-developed and showed no signs of abnormality. When he was 1—2 years old the parents noticed that he had a squint in his right eye. His eyes were not examined by a specialist, however, until he was 5 years of age. It was then found that there was a healed chorioiditic lesion in the squinting eye; this eye was also slightly smaller than the other one. The infant was examined at the Sachs' Hospital for Children in June 1950, but no other signs and symptoms were observed

except for those from the eyes. A serologic test on both mother and infant, with a view to demonstrating the presence of toxoplasmosis, gave a positive result. This finding, in conjunction with those from the eyes, made the diagnosis of congenital toxoplasmosis seem probable in this case.

Date	Dye test		Complement fixation reaction		Toxoplasmin skin test		Other data
	Mother	Infant	Mother	Infant	Mother	Infant	
20.9.44	—	—	—	—	—	—	Partus
21.1.50	—	1: 250	—	—	—	—	—
11.2.50	1: 50	1: 50	—	—	—	Pos.	—
1.3.50	1: 50	1: 50	—	—	—	—	—
20.4.50	1: 50	1: 250	1: 24	1: 16	—	—	—
30.8.50	1: 50	1: 50	1: 24	1: 30	—	—	—

Case 4. The mother, aged 22, was a primipara, and showed no signs of illness. She was examined at regular intervals during her pregnancy, which was uneventful.

The infant, a girl, was born at the expected time, after normal delivery, and weighed 2 900 g. She was well developed. Examination of the eyes at the age 4 months revealed a healed peripheral chorioiditic lesion in the right eye. An examination carried out at the Sachs' Hospital for Children in October 1949 brought out nothing of interest except the symptoms from the eyes. X-ray of the head yielded no information. Both physically and mentally, the infant showed normal development. No other signs or symptoms were observed at subsequent examinations. A serologic test, performed with a view to demonstrating toxoplasmosis, yielded a high titer for both mother and child. This finding, in conjunction with the eye manifestations, provided strong evidence in favour of the diagnosis congenital toxoplasmosis in this child.

Date	Dye test		Complement fixation reaction		Toxoplasmin skin test		Other data
	Mother	Infant	Mother	Infant	Mother	Infant	
8. 2.49	—	—	—	—	—	—	Partus
20. 9.49	1: 500	1: 1 000	—	—	—	—	—
9.10.49	1: 500	1: 1 000	—	1: 8	—	Pos.	—

Case 5. Father aged 40, healthy. Mother aged 35, a secondipara, healthy. She was examined at regular intervals during pregnancy, and nothing eventful occurred.

The infant, a girl, was born a month before term and weighed 2 100 g. She was well developed, and showed no signs of illness at first. At the age of 10 months a squint in the right eye was discovered. Examination of the eyes by an ophthalmologist revealed a healed chorioiditis lesion, with atrophy and pigmentation, and hypermetropia of the squinting eye. A serologic test, aiming at demonstrating toxoplasmosis, showed a positive result, and the patient was admitted to the Sachs' Hospital for Children for observation. Multiple intracranial calcifications were now detected. These clinical signs and symptoms, together with the serologic findings, justified the diagnosis of congenital toxoplasmosis in this patient.

Date	Dye test			Complement fixation reaction			Toxoplasma skin test			Other data
	Father	Mother	Infant	Father	Mother	Infant	Father	Mother	Infant	
23.3.45	—	—	—	—	—	—	—	—	—	Partus
21.3.50	1: 50	1: 50	1: 250	1: 24	1: 24	1: 60	—	—	—	—

Case 6. A 20-year-old woman who had had measles, whooping cough and German measles when a child but had otherwise enjoyed good health. Her husband had also been healthy. No miscarriages. She became pregnant in September 1947 and was examined regularly at a Women's Health Centre; she felt quite well during her pregnancy and displayed no signs of illness. On June 7th, 1948 she gave birth to a female infant three weeks before term. The weight of the infant at birth was 2 450 g, the length 46 cm and the circumference of the head 29 cm.

The infant, after a breech delivery, was deeply asphyxiated. After aspiration of mucus and allantoic fluid, the infant began to cry, but a short time afterwards generalized convulsions set in and it became cyanotic and hypotonic. In spite of energetic measures it died 20 minutes after birth. The placenta appeared to be normal, judging from the usual inspection.

Autopsy (Dr F. Wahlgren). When the abdomen was opened, the stomach and the entire small intestine, the spleen and the left lobe of the liver were found in the left pleural cavity. These organs have slipped up through a slit of several fingers' breadth between the lateral and medial lumbocostal arches of the diaphragm. From the left pleural cavity, parts of the organs were bulging out into the right pleural cavity. The heart and great blood vessels displayed no abnormality. The left lung was small and generally atelectatic. The right lung was also smaller than normal, the alveolar lung substance being pale and scanty. Neither

Date	Dye test		Complement fixation reaction		Toxoplasmin skin test		Other data
	Mother	Infant	Mother	Infant	Mother	Infant	
7. 6.48	A rabbit skin test was performed with a blood sample from the mother, with a negative result.						Premature partus (infant died of diaphragmatic hernia 7.6.48)
23. 9.48	A dye test was carried out on the blood sample taken from the mother on 7.6.48, titer 1: 3 200.						
16.11.48	1: 600	—	—	—	—	—	
15. 1.49	1: 600	A complement fixation reaction, using the blood sample taken from the mother on 7.6.48, was positive in a dilution of 1: 12.					
24. 3.49	1: 500	—	—	—	—	—	
19. 5.49	1: 250	—	—	—	—	—	
1. 9.49	1: 250	1: 250	—	—	—	—	Normal partus (Birth weight 3 700 g. Infant observed 1 year without symptoms)
20.10.49	1: 250	1: 50	—	—	—	—	
18. 7.50	1: 50	Neg.	1: 6	—	Pos.	—	

trachea nor bronchi contained foreign substances. The greater part of the liver and the descending colon were all that were left in the abdominal cavity. The liver was deformed and clumsy, and the cut surface was dark reddish brown. The bile ducts were normal. The stomach and parts of the small intestine were filled with gas. The spleen was of normal size and consistency, and the cut surface was dark red. Adrenal glands normal. Kidneys, urinary passages and genital organs normal.

A blood sample was taken from the mother on the 7th and from the father on the 8th of June, and a skin test was performed on rabbits. The result was negative in both cases. The serum sample from the mother was preserved at a temperature of —60° C. A little over three months later, on September 23rd, this sample was re-examined with the Sabin-Feldman dye test. A titer of 1:3 200 was obtained, and the mother was therefore kept under observation.

In December 1948, after having been in good health in the interim, she became pregnant again. Her last menstrual period was between

Dec. 4th and Dec. 8th, 1948. The pregnancy was quite uneventful, and the patient was kept under observation by her physician. She gave birth on Sept. 1st, 1949, at the expected time, and after a normal and easy delivery, to a healthy male infant weighing 3 700 g. This infant has been under observation for over a year. It has developed normally and shown no signs of illness. With regard to the serologic tests to demonstrate toxoplasmosis, the results are shown in the table.

Discussion

The unequivocal cases of prenatal toxoplasma infection reported in the literature all seem to have been congenital, in other words, the pregnancy ran its full course, or very nearly so, even in those cases where the infection led to the intrauterine death of the fetus. The question of whether toxoplasmosis can lead to abortion has aroused some discussion, and various opinions have been advanced. While the present paper is not intended to be a contribution to this debate, we would mention here that we have observed a case which demonstrated that toxoplasmosis can be a cause of abortion. The case in question, which is to be described in another connection, was that of a woman who was examined in connection with a spontaneous abortion in the third month. A serologic examination at the time of the abortion showed a dye test titer of 1: 40. A toxoplasma strain typical in all respects was isolated from the aborted remains. At a second serologic examination five weeks after the abortion, the titer had risen to 1: 250. The same value was obtained eight weeks later, but after 17 weeks the titer had dropped to 1: 50. The demonstration of organisms, and the typical serologic findings, would seem to be a proof that the diagnosis was correct in this case.

The question of whether toxoplasmosis causes congenital deformities is also open to discussion. In case 6, in which the infant had a large diaphragmatic hernia at birth, a complete investigation was not possible owing to several unfortunate circumstances. In the first place, the cause of the infant's death was considered to be intracranial hemorrhage due to injury in connection with the extraction, and the physician in charge was so strongly convinced of this that it was considered unnecessary

Table I.

Dye test values in the mothers of infants with diaphragmatic hernia.

Case	Date of birth (infant)	Date of test on mother	Dye test	Comments
1	31. 5.1939	27. 1.1949	1:40	Brain not exmined
2	23.11.1945	18. 2.1949	1:25	»
3	6. 7.1946	27. 1.1949	Neg.	
4	11.12.1946	Dec. 1948	Neg.	Twin
5	11. 3.1947	21. 1.1949	Neg.	
6	16. 4.1948	11. 1.1949	1:20	Brain normal

to open the head at the postmortem examination. Nor was any material collected for a microbiologic examination. In the second place, the first serologic test on the mother's serum (intracutaneous neutralization in rabbits) showed a negative result, and it was not until over three months later, when a dye test was carried out on the same serum, that toxoplasmosis was suspected in this case. Every possibility of studying the case in more detail had by that time been lost. It is therefore impossible, now, to decide whether a toxoplasma infection of the fetus had occurred, and if it was, whether the infection played any part in producing the diaphragmatic abnormality. In any case, it would seem to be fairly well established that the mother passed through an infection either during, or a short time before, her pregnancy.

In view of this observation, serologic tests were carried out on the mothers of six other previously observed infants that had died within a few days of birth as the result of diaphragmatic hernia, all of them with extensive deformities. The results are shown in table I.

As may be seen from the table, a period of between 9 months and 10 years had elapsed from the birth of the respective children to the date of the tests on the mothers. In no case was a high titer value, indicating a relatively recent process, obtained. The incidence of positive reactions (50 per cent) is in complete accord

with that obtained in a series of normal adult women in Stockholm. This suggests that prenatal toxoplasmosis probably cannot be regarded as the cause of malformations of this kind.

One of the details observed in connection with this case is worth mentioning. The serum that gave a negative result in the intracutaneous neutralization test but a dye test titer of 1: 3 200 showed an uncommonly strongly pronounced negative zone when a complete titration was carried out. Even in the 1:40 dilution considerably more than 50 per cent of the toxoplasma were unchanged both in shape and stainability, and in dilutions below 1: 10 practically no serum effect was detectable, a fact which furnishes a very probable explanation of the negative neutralization reaction. This observation shows clearly one of the weaknesses of the neutralization test.

The other cases described in this paper require no special comment. Case 1 is in complete agreement with the classical picture of congenital toxoplasmosis, and the diagnosis in this case was established with absolute certainty by the demonstration of the infective agent both microscopically and in animal experiments. In cases 2—5, the etiologic diagnosis was based wholly on the results of the serologic tests. However, in view of the fact that positive reactions were never encountered in the age classes in question in our material except when chorioretinitis or other signs of congenital toxoplasmosis were simultaneously present, the diagnosis of toxoplasmosis may be regarded as highly probable in these cases also.

Summary

After a description of the classical syndrome in congenital toxoplasmosis five cases encountered by the writers are presented. In one of these patients, who was observed during the active stage of the process, toxoplasma were demonstrated in the ventricular fluid. This case displayed all the typical signs and symptoms of the disease. The other cases displayed varying degrees of severity, and the diagnosis in these patients is based on the results of the serologic examinations. In an unequivocal case of toxoplasma infection (asymptomatic) in the mother, a large diaphragmatic hernia was observed in the premature infant. Unfortunately, this case was not investigated satisfactorily, and it is not

possible to make any statement regarding the cause of the abnormality. A follow-up investigation on six mothers of infants with diaphragmatic hernia failed to yield evidence in support of the theory that toxoplasmosis might be a cause of such malformations.

Toxoplasmose congénitale.

Après la description du syndrome classique de toxoplasmose congénitale, les auteurs présentent 5 nouveaux cas observés par eux-mêmes. Chez l'un de leurs malades, qui fut étudié pendant la phase active de la maladie, la présence du toxoplasme fut mise en évidence dans le liquide ventriculaire. Ce cas présentait tous les signes typiques de la maladie. Les autres cas, caractérisés par des atteintes de sévérité variable, furent diagnostiqués de façon certaine grâce aux examens sérologiques. Dans un cas d'infection à toxoplasmes dont la réalité ne peut être mise en doute (forme asymptomatique) chez la mère, une hernie diaphragmatique importante fut retrouvée chez l'enfant né prématurément. Malheureusement, cette observation ne put être fouillée suffisamment, et, il ne nous est pas possible de formuler une conclusion définitive quant à l'étiologie de cette malformation congénitale. Un étude faite sur six mères d'enfants porteurs de hernie diaphragmatique ne vient pas à l'appui de la théorie qui met en avant la toxoplasmose comme pouvant être la cause de semblables malformations.

Kongenitale Toxoplasmosis.

Nach einer Beschreibung des klassischen Syndroms bei kongenitaler Toxoplasmosis teilen die Verfasser fünf von ihnen studierte Fälle mit. Bei einem dieser Patienten, welcher während des aktiven Stadiums des Prozesses beobachtet wurde, wurde Toxoplasma in der Ventrikelflüssigkeit nachgewiesen. Dieser Fall wies alle typischen Zeichen und Symptome der Krankheit auf. Die andern Fälle zeigten wechselnde Schweregrade, und die Diagnose gründete sich bei diesen Patienten auf die Ergebnisse der serologischen Prüfungen. In einem unzweideutigen Fall von (asymptomatischer) Toxoplasmainfektion bei der Mutter wurde ein grosser Zwerchfellbruch bei dem vorzeitig extrahierten Kinde beobachtet. Leider wurde dieser Fall nicht genügend untersucht, und es ist nicht möglich, etwas über die Ursache der Anomalie zu sagen. Eine Untersuchung bei sechs Müttern von Kindern mit Zwerchfellbruch lieferte keine Stütze für die Theorie, dass Toxoplasmosis eine Ursache solcher Missbildungen sein kann.

Toxoplasmosis congénita.

Tras una descripción del sindrome clásico de la toxoplasmosis congénita se presentan 5 casos hallados por los autores recientemente. Uno de ellos que fué observado durante el estadio agudo de la enfermedad tenía toxoplasmas identificables en el líquido ventricular presentando todos los síntomas y signos típicos de la enfermedad. Los casos restantes en los cuales la enfermeded presentaba grados variables de severidad, el diagnóstico estaba basado sobre los resultados de los exámenes serológicos. En un caso indudable de infección toxoplasmósica asintomática de la madre, en un niño prematuro que tuvo pudo comprobarse una amplia hernia diafragmática, desgraciadamente este caso no pudo investigarse de un modo completo satisfactorio y no es posible afirmar de un modo estricto la causa de esta malformación. La investigación subsiguiente de 6 madres de niños con hernia diafragmática no mostraba evidencia para suponer la teoría de que la toxoplasmosis pudiera ser la causa de tales malformaciones.

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Studies on Carbonic Anhydrase Activity in Children

1. Enzyme Activity in the Blood of Infants and Children of Different Ages, particularly in Premature Infants

by

RAGNAR BERFENSTAM

Carbonic anhydrase (C.a.) is an enzyme whose function it is to serve as a catalyst for the formation and decomposition of carbonic acid. As such it plays a very important rôle in the transport of carbonic acid in the blood and tissues and thus intervenes in the total acid-base equilibrium. The enzyme is a *zinc proteid*, containing about 0.3 per cent zinc (15) and about 15 per cent nitrogen (16). The molecular weight lies around 30 000. The qualitative amino acid composition is not definitely established as yet. C.a. is a very active enzyme; its activity is still perceptible at concentrations as low as 1: 10 000 000.

C.a. is found not only in mammals but also in lower animals (12). In plants an enzyme closely related to carbonic anhydrase is believed to be present (9).

After the fundamental studies of HENRIQUES[1] on the binding of carbonic acid in the blood it became clear that for the carbonic acid turnover to take place at a sufficient rate, the presence of an enzyme was necessary (13). One would have been otherwise unable to explain how the blood could free itself from carbonic acid in the short time it remained in the lung capillaries. If the reaction $H_2CO_3 = H_2O + CO_2$ were to take place without

[1] For further references on earlier litterature see H. VAN GOOR: Carbonic Anhydrase, its properties, distribution and significance for carbon dioxide transport. Enzymologia *13*, *73*. 1948.

a catalyst only a small percentage of the carbon dioxide would be liberated. Some elucidating investigations on this subject were carried out by BRINKMAN, MAGARIA, MELDRUM and ROUGHTON in 1932 (7). The two last named scientists soon afterwards succeeded in showing that the enzyme concerned with this reaction was a substance different from hemoglobin which they called carbonic anhydrase (21).

The enzyme has since been the subject of intensive investigations. It was shown that, like other enzymes, it was affected by activators and inhibitors. Certain amino acids, such as cystine, and cysteine as well as glutathione and extracts from animal organs function as activators (18), whereas cyanides (22), carbon monoxide (22), heavy metals (22) and certain sulfonamides (10) are strong poisons. The presence of activating and inhibiting substances in the plasma of certain mammals was also demonstrated (5, 18).

In mammals the enzyme is present in the blood as well as in most internal organs. In the blood, the enzyme is found in the red cells, but none is present in the plasma, the urine or milk. Among the internal organs, the highest activity is found in the gastric mucosa, kidney, pancreas, testis and brain (12).

From the distribution of the enzyme in the organs it would seem that it should have some other physiological functions besides carbonic acid turnover in the blood. PITTS (23) has thus elaborated a theory according to which C.a. is of great importance for the acidification of the urine in the tubules. He bases this on the observation that the enzymic poison sulfonamide causes the production of a more alkaline urine and also an acidosis when administered to experimental animals. Clinical experience with sulfonamide therapy confirms these observations. The most recent investigations have thrown doubt on the earlier held supposition that C.a. also regulates the hydrochloric acid secretion in the stomach (8).

There are comparatively few clinical papers on C.a. and most of them concern themselves with the enzyme content of the blood. It was found in general that the carbonic anhydrase activity in the blood of healthy adults varied little from case to

case and further that the enzyme activity in the same person remained practically constant in repeated determinations (12).

The values for the enzyme follow, in general, the hematocrit value (14). Thus patients with a serious heart ailment with cyanosis and polycythemia have a higher C.a. content in blood, whereas patients with anemia have a lower one. Some exceptions are pernicious anemia and hepatic disease, which give rise to a higher value of the enzyme in relation to the hematocrit value (17, 19, 29).

LAMBIE (17) investigated the enzyme content of blood in pathological conditions in man and found one case (black water fever) with only 1/5 of the normal enzyme activity, without it being possible, however, to demonstrate any alteration in the chemical composition of the blood or in the amount of CO_2 given up. This finding is in agreement with the calculations of ROUGHTON (26), according to which there normally is a very *great excess of carbonic anhydrase* in the body.

In 1940, two German scientists, SCHMITT and FRIGGE (27) found that intravenously administered C.a. had an effect on acid-base equilibrium.

MELDRUM and ROUGHTON (22) had already shown in 1933 that the blood of the fetus of the goat had a very low carbonic anhydrase activity. In analogy to this, STEVENSON, in 1943 (28), showed that premature infants also had a very low activity, a finding which made this enzyme become of pediatric interest. Stevenson also thought that attacks of cyanosis were most common in infants with a low enzyme content. DAY and DU PAN (11) have verified Stevensons investigations concerning the low C.a. content, but have also shown that this does *not* seem to have any influence on the acid-base equilibrium of the body.

Purpose of the investigation

The purpose of the first part of this investigation was — starting from the earlier established fact that the carbonic anhydrase activity in the blood of premature infants is low — to study closely a greater number of infants in this respect in order

to look for a relationship between the enzyme activity and the degree of prematurity and also to follow the changes in activity during the first months of life.

Methods

The enzyme analyses in this investigation were performed according to the manometric technique of ROUGHTON (24), i.e., the so-called "boat method." The principle of this procedure can be shortly described in the following manner: A solution of phosphate buffer and a solution of bicarbonate containing the enzyme sample are introduced into a reaction vessel divided into two compartments by means of a ridge in the bottom. The two solutions are rapidly mixed by shaking the vessel, and the CO_2 liberated is measured every five seconds by means of a manometer. The same procedure, but without the enzyme solution is repeated in order to obtain a blank. By plotting the time against the manometer readings one obtains two curves: one shallow one for the blank and one steeper one for the experiment proper (Fig. 1). After some time the curves will coincide. The unit of enzyme activity is a mathematical expression for the increase in rate and is calculated according to the original formula of Roughton: $E = \frac{R - R_0}{R_0}$, where R is the velocity of the second quarter of the reaction in the presence of the enzyme and R_0 the corresponding velocity for the blank.

Some modifications of the original method of Roughton have been introduced here.

1. The compensating vessel, which according to Roughton consisted of a bottle placed on the bottom of the water bath was substituted by a flask of the same size and shape as the reagent vessel. Both flasks were shaken in the same holder. This setup has the advantage that the reagent vessel and the control are subject to exactly the same conditions.[1] Ground glass joints were used for the reagent vessel and thick vacuum tubing to connect the flasks with the manometer.

2. Shaking was carried out around a vertical axis at a rate of about 360 r.p.m.

3. Since, for practical reasons, the manometer has to be placed outside the water bath, the reaction temperature has been chosen at 20° C in order to eliminate large temperature variations between the manometer and the rest of the apparatus. (The importance of this is also pointed out by LAMBIE (17).) The activity unit is then going to be disignated as E^{20}. Roughton has generally used a reaction temperature of 0 or 15° C.

[1] This modification was later also introduced by ROUGHTON and BOOTH (25).

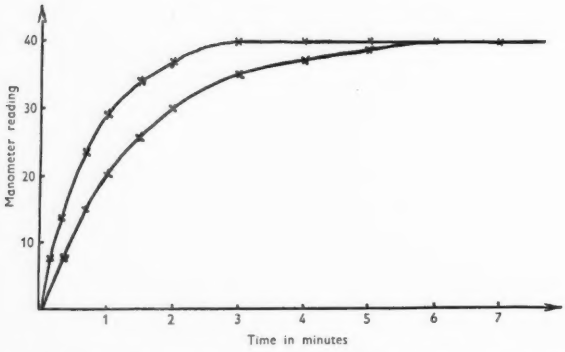

Fig. 1. *Diagram of the course of an enzyme analysis.* The upper curve represents the enzyme sample and lower one the blank.

For the blank a sample inactivated by heating at 65° for 10 minutes has been used instead of the active enzyme sample (in this case hemolysed blood in great dilution). This was done in order to insure greatest uniformity in conditions concerning salt concentration and other similar factors. The same results were obtained, however, when the blank was carried out with water only.

Duplicate determinations were made for each enzyme solution. When a satisfactory agreement was not obtained, the analyses were repeated again. It was in general sufficient to control the blank only a few times a day, since the values always agreed if the same buffer and bicarbonate solutions were used, and the latter kept in the cold. The experimental error, calculated from duplicate determinations was found to be $< \pm 5$ per cent.

Taking of the sample:

This investigation concerned itself only with enzyme determinations in the blood. Capillary blood, 0.01—0.025 ml, was taken from the fingertip or the heel without stasis. This was hemolysed immediately with distilled water. In some cases, when venous blood was available it was also used for the determinations, and it was always found that the values were the same as for capillary blood.

It was found that only at relatively low concentrations was there a direct proportionality between concentrations and activity. At higher concentrations the corresponding activities are to low as is seen from Fig. II. This behaviour has earlier been pointed out by LAMBIE (17). If then, one expects a high activity, the sample should be hemolysed in a larger volume of water than if the activity is expected to be low.

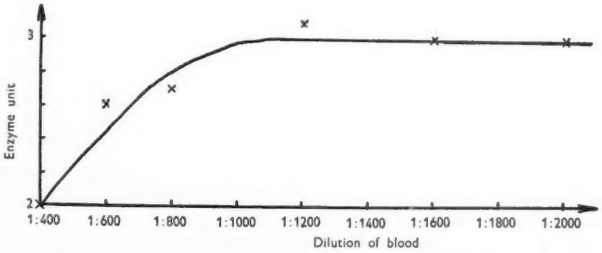

Fig. II. *Relationship between the enzyme activity and concentration in analyses performed on the same blood sample in different dilutions.*

It was generally found suitable to dilute the blood from adults 1: 1.200, that of newborn infants 1: 800, and that of premature infants 1: 200.

In the hemolysed state the samples could be kept in the cold as long as 14 days without loss of activity. Most determinations have, however, been performed within 5 days. The activity also did not change if venous blood was kept with heparin in the unhemolysed state.

Results

A total of about 2 000 analyses were performed in the years 1947—48. In order to study the carbonic anhydrase activity in newborn infants, it was necessary to obtain a normal value. This was based on determinations on 63 adults. The material was further composed of 33 premature infants, 39 fullterm infants and a number, 101, of healthy children in different ages.

Activity of C.a. in the blood of adults, children and infants

The material for the normal value was obtained from adult men and women of 18 to 93 years of age. The average value was found to be $E^{20} = 2.75 \pm 0.41$. The spreading of the results was small, a behaviour which had already earlier been observed.

The fact that the activity in the same person remains relatively constant can be seen from Table I, which represents repeated determinations in the blood of some adults. Newborn fullterm infants show a considerably lower activity than adults. An average value for these infants was found to be $E \equiv 1.0 \pm 0.44$.

Table I.

Repeated determinations of enzyme activity in the blood of some adults.

Date	Name	Enzyme Activity Unit/mm³
3.8. 48	K.R.	3.1
13.8. 49		3.2
3.8. 48	S.S.	3.1
13.1. 49		3.2
3.8. 48	A.S.	3.1
13.1. 49		3.2
7.3. 49		3.8
23.5. 49		3.0
26.1. 49	E.W.	3.6
23.5. 49		3.5
20.7. 49		3.6

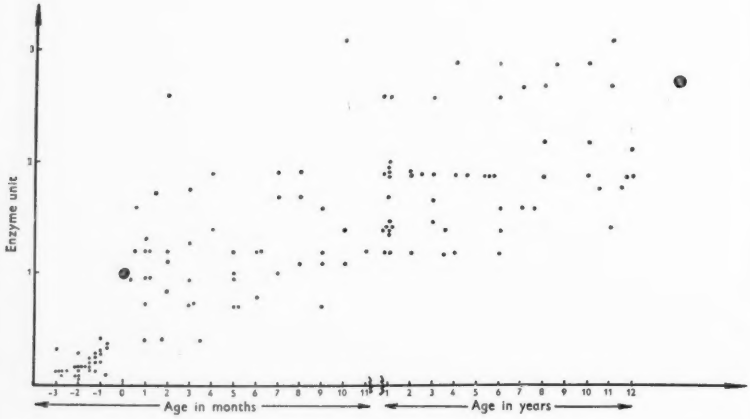

Fig. III. *Diagram of the relationship between enzyme activity and age.* The scale along the x-axis is interrupted and represents age in months for infants less than one year old and age in years for children between one and twelve years of age. The large circles represent average values for newborn infants (39 cases) and for adults (63 cases).

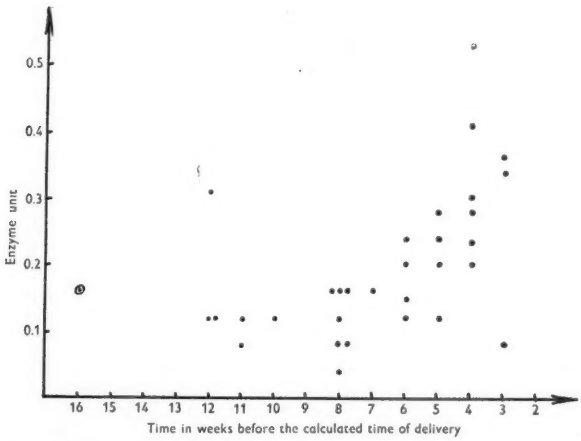

Fig. IV. *Diagram of the enzyme activity in premature infants.*

The enzyme activity was also determined on blood from the umbilical cord and the values were found to be consistent with the others.

A mean value for the activity in premature infants can not be given as this group is heterogeneous regarding maturity. The highest value obtained for a premature infant is 0.50 E.

From determinations in children of various ages, it was found that *the activity increase with increasing age.* The results are shown by the diagram in Fig. III. The average values from adults and newborn infants are introduced as points of reference. The results obtained from premature infants are also shown in the same diagram. It can be seen clearly, that the activity in premature infants is very low, even in relation to newborn fullterm infants, and that the activity increases with increasing development. This latter can be seen from the diagram in Fig. IV. The results given here refer to samples taken during the first two days of life for the premature infants and during the first week of life for newborn fullterm infants.

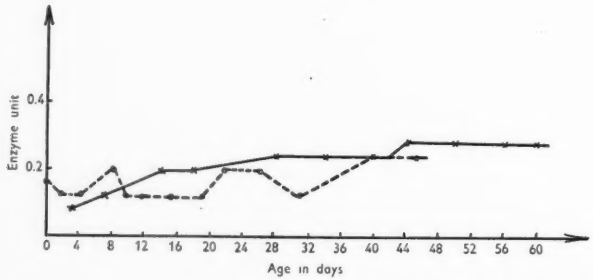

Fig. V. *Enzyme activity in the blood of two premature infants during their stay in the hospital.* The full line represents the infant M–z (Ref. 895/47, weight at birth 1 900 g.) and the dotted one the infant B–l (Ref. 429/48, weight at birth 1 800 g.).

Carbonic anhydrase activity in premature infants during the first months of life

The activity of the enzyme in premature infants was studied during their period in hospital. As an example two of the activity curves have been introduced in Fig. V. During the first month of life one can observe a slow increase in activity despite the fact that the hematocrit value, i.e., the total volume of red blood corpuscles (the carriers of the enzyme), decreases during this time. Irregularities in the course of the curve could perhaps be due to variations in the fluid equilibrium which is a relatively common occurrence in these infants. In a few cases determinations of activity were carried out after the premature infants had reached a higher age. No definite difference from the activity of infants of the same age but born at full term could be ascertained, however.

Instead of giving the activity in terms of whole blood, it could be given in terms of red blood corpuscles, since these contain all of the enzyme. An expression for the activity in the red blood cells, can be obtained by dividing the whole blood activity by the hematocrit value. In the later part of this determination the hematocrit value has therefore always been determined and the above mentioned ratio calculated. A diagram representing the relationship between these ratios and the degree of develop-

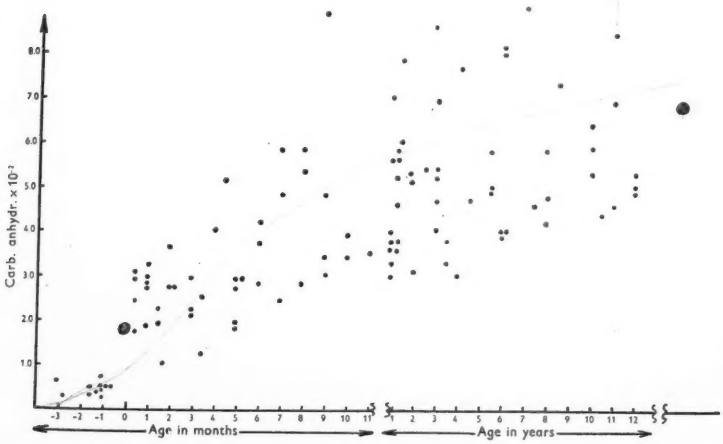

Fig. VI. *Diagram of the relationship between the age and enzyme activity* (expressed as the quotient between enzyme unit and hematocrit value).

ment of the infants can be seen in Fig. VI. The agreement between this diagram and that of the whole blood seems good.

For this investigation the enzyme activity of whole blood must be of greater value, however, since it represents the actual activity available to the organism.

Discussion

The values for the carbonic anhydrase activity in adults as found by different investigators differ appreciably from each other as is seen from table II. That might be due to differences in the apparatus used. It is of the greatest importance, however, that all determinations in a certain investigation be carried out under exactly the same conditions in order to permit comparison of the results.

The value for adults, found by the present author is of the same order of magnitude as that of STEVENSON. As mentioned earlier, STEVENSON has also shown that premature infants have a very low C.a. activity. His value is, however, appreciably higher than the value found in the present investigation. That

Table II.

Carbonic anhydrase activity in blood of adults, according to different authors.

Author	Unit/mm³	Temperature
Meldrum and Roughton ..	0.37—0.68	15°
Hodgson	1.39—1.75	15°
Lambie	1.25—1.50	15°
Stevensson	3.5	0°
Berfenstam	2.75	20°

might be due to the fact that STEVENSON used the same dilution for the bloods of both adults and premature infants, which, as shown, can lead to a lowering of the sensitivity of the method.

Recently DAY and FRANKLIN (10) published an investigation concerning the C.a. activity in kidneys from premature infants. These authors found that the value for C.a. activity in blood from premature infants was about 10 per cent of the adult value.

ASHBY (3) studying the C.a. activity in the brain of premature infants gives values for C.a. activity in blood in these infants in agreement with those of the present author.

Other results are reported, however. In 1950, after the experimental part of the present investigation was finished, ALTSCHULE and LEWIS published a somewhat different method for the determination of carbonic anhydrase activity (1). The new method was used by ALTSCHULE and SMITH (2) in a clinical investigation of the enzyme content in the bloods of mother and infant. The main difference of this method from the earlier used "boat method" of ROUGHTON is that the reaction is allowed to proceed at 37° C in a Warburg apparatus using a slower rate of vibrations than is costumary with the "boat method". Also for the calculation an other method than that of ROUGHTON was used.

From the results of the investigation of ALTSCHULE and SMITH it appears that the activity in newborn premature infants is, in fact, lower than that of the mothers, but that the difference

is moderate (about 60 per cent lower) in comparison to the great difference found in the present investigation.

According to their findings, ALTSCHULE and SMITH are apt to believe, that the difference in activity between mother and infant is of little physiological importance. The authors also show that the new method eliminates only *some* of the factors that made the previous method unphysiological.

It is impossible for the present author to evaluate this change in the method of analysis and calculation. In investigations like the present, however, where the objective is to compare values obtained under similar conditions from different individuals, (i.e., to compare the values in infants with those of adults) it should be possible to make comparisons, even if the experimental conditions were unphysiological but satisfactory otherwise.

Correlation between enzyme activity and degree of development

STEVENSON's values show a considerable spread also in the case of premature infants (0.00—1.40 E), but the spread is particularly great in full term newborn infants (0.00—1.90). Several of these last-named values are at the same level as the values for premature infants. It is of special interest that in premature, as well as in full term infants, there are in Stevenson's investigation cases in which no activity can be ascertained. The results given thus are difficult to evaluate.

From the diagram in Fig. IV a correlation between the enzyme activity and the degree of maturity is clearly apparent. The deviating values can, in certain cases, be explained by incorrect information about the duration of the pregnancy. A clear example of such a case is represented by the encircled value of the diagram where, according to the mother, the child was born 16 weeks too early; the infant was, however, 41 cm long and weighed 1.800 g, clearly indicating the incorrectness of the statement, which could also be seen from the activity value.

It is known that in many cases the length and more particularly the weight at birth is not proportional to the degree of prematurity. A diagram of the enzyme activity first in relation

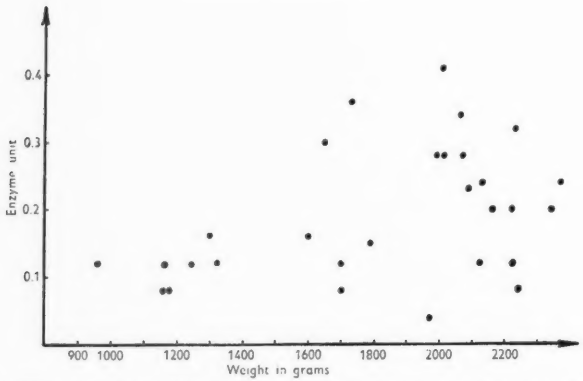

Fig. VII A. *Relationship between enzyme activity and weight at birth in premature infants.*

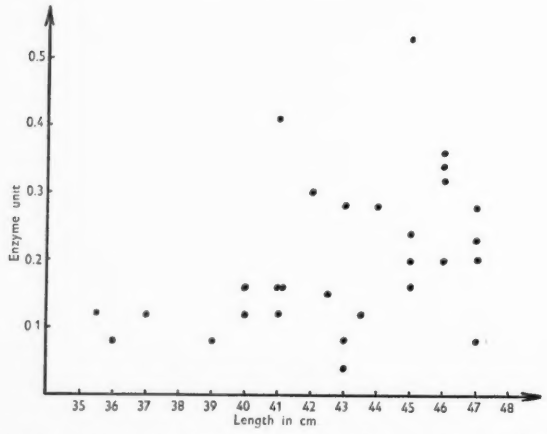

Fig. VII B. *Relationship between enzyme activity and length at birth in premature infants.*

to weight and then in relation to length at birth is shown in Fig. VII a and b. It can be seen that there is a relatively large spreading of the correlation values between weight and enzyme activity, whereas the correlation between activity and length is somewhat better.

Table III a.

Enzyme determination in the blood of full-term infants with low weight at birth.[1]

No.	Name	Weight at Birth in g	Enzyme Unit/mm³
1015/48	B—j	1 960	1.7
63/49	J—f	1 950	1.0
367/49	E—n	2 000	0.8
969/49	S—n	1 350	0.9
1138/49	N—n	2 350	1.0
286/49	L—n	2 300	0.8
49/49	N—g	1 950	0.9

Table III b.

Enzyme determination in the blood of premature infants with a normal weight at birth.

No.	Name	Weeks before expected date of delivery	Weight at Birth in g	Enzyme Unit/mm³
296/49	P—n	5	2 700	0.4
211/49	K—n	3	3 350	0.4

It happens sometimes that one finds a very low weight at birth even though, according to the mother, the infant was born at the calculated time. Since these statements always have to be accepted with reservation, some activity determinations were made on a number of such infants. The results, shown in table III a show the interesting fact that the enzyme activity is normal and does not correspond to the "premature" weight. One evidently has to deal with fully developed, but underweight infants. The

[1] For comparison: Average value for enzyme activity
in newborn full-term infants: 1.0
in newborn premature infants: < 0.50.

same is often true for twins who, although born in time, weigh very little but show a normal C.a. activity.

The contrary is also found in the case of diabetic mothers among others, where infants with as high a weight at birth as full term infants were prematurely born, according to the mothers. Some of these cases have been given in Table III b, which shows that the activity values clearly deviate from those of full term infants.

Since then, the activity increases rapidly during the last part of embryonic development, the C.a. activity should form a good criterium for the degree of development. The question arises, however, whether the activity continues to rise when the normal duration of pregnancy is exceeded. Only one such a case was investigated, and a higher value than normal was obtained.

Thus, this investigation supports the experience, that it is often better to judge the viability of a newborn infant according to the pregnancy duration (if the statement can be trusted) instead of relying upon the weight.

Some view points on the cause and clinical importance of the low enzyme activity in premature infants

It is difficult to explain the low enzyme activity in the blood of premature infants. It would be easiest to suppose that the enzyme is mainly formed during the last months of embryonic life. Another explanation would be the assumption that some activating substance present in the blood of adults is lacking in that of the infants. Other variations in the equilibrium between activators and inhibitors such as exist in adults are naturally also possible. According to the calculations of Roughton there should be such an excess of C.a. in adults that perhaps only as little as 1/100 of the total amount is necessary fot the catalysis. One should suppose, then, that the low enzyme value in newborn premature infants would have no great significance. The gas exchange in the organism, however, depends partly on the rate of the exchange reaction, but partly also on other factors, such as the histological structure, vascular supply and state of

function of the lungs. In this latter respect premature infants are not well equipped: the atelectases take up a lot of pulmonary space, the alveoli are less in number and the capillary network is as yet sparse and coarse. These imperfections contribute to make the gas exchange more difficult and make therefore a great demand on the reaction rate.

If it is true that within certain limits the reaction rate is proportional to the enzyme activity, then, since these premature infants have only about 1/10 of the activity of adults, it is possible that this low enzyme content contributes to the infant's difficulty in having a proper gaseous exchange.

Therefore, the question about the relation between the low carbonic anhydrase content of the blood of premature infants and their tendency towards cyanosis, put forwards by STEVENSON, is still open. Even if the majority of the cases of "unexplained cyanosis" described by STEVENSON has less then 2/3 of the C.a. activity of normal premature infants, and even if most of these cyanotic infants weigh less than 1 600 g at birth, the variations in the enzyme activity in his material is so large, that it is difficult to draw any reliable conclusions from this "depression". In the material for the cases cited as "unexplained cyanosis", full term infants with an almost normal enzyme content have also been included while, on the other hand, there are full term as well as premature infants without cyanosis, but in whose blood no activity at all could be found.

In the author's material there is no obvious correlation between cyanosis and a remarkably low enzyme activity. It is shown, however, as already is pointed out, that there is a good correlation between enzyme activity in blood and the degree of maturity. Perhaps the reduced vitality, often seen in the most prematurely born infants, to *some* extent may be sequelae of the difficulties in the gas exchange due to the low activity in carbonic anhydrase.

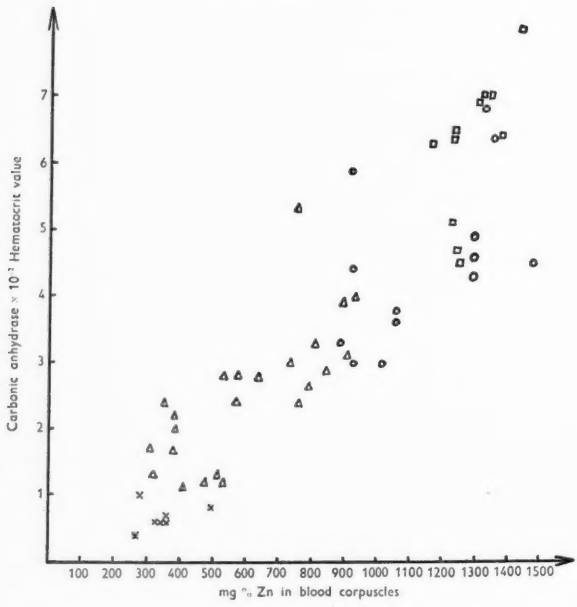

Fig. VIII. *The Relationship between Carbonic anhydrase and zinc in blood from infants, children and adults.*

× = premature infants
△ = umbilical cord and full-term infants
○ = children
□ = adults

Correlation between zinc content and carbonic anhydrase activity in the blood

In an earlier investigation the connection between C.a. activity and zinc content of the blood corpuscles has been discussed (4). Carbonic anhydrase is, as pointed out earlier, a zinc proteid. The average zinc content of the blood corpuscles of adults was shown to be about 1 300 μg per cent, whereas the value for newborn infants lies around 500 μg per cent and the value for premature infants is still lower. It would seem then that there is a correla-

tion between the zinc content of the red blood corpuscles and the carbonic anhydrase activity.

This relationship is also apparent from the diagram in Fig. VIII where the enzyme activities and the zinc content of the blood of some premature, some normal newborn infants and some children of different ages are represented.

Summary

1. A short review of the literature on the enzyme carbonic anhydrase is given. Special weight has been laid on earlier clinical work.

2. An account of the author's method, which is a modification of the "boat method" of MELDRUM and ROUGHTON, is given.

3. A number of normal values for the C.a. activity in the blood of adults, children in various age groups and infants are given. It is concluded, that the activity for new-born infants is about one third that of adults, and that the enzyme activity in infants and children increases with increasing age.

4. The enzyme activity in the blood of premature infants is shown to be only about 5—10 per cent that of adults. The activity increases slowly during the first months of life.

5. The relationships between the enzyme activity and the degree of maturity, length and weight at birth are discussed. There seems to be a good correlation between enzyme activity and maturity.

6. The clinical importance of the low enzyme activity for cyanosis and vitality in premature infants is discussed. The author has not been able to verify the finding of STEVENSON, that cyanosis often is associated with low carbonic anhydrase activity.

7. A parallel relationship between the enzyme activity and the zinc content of the blood is demonstrated.

Études sur l'activité de l'enzyme anhydrase carbonique chez l'enfant: 1. Activité enzymatique dans le sang d'enfants de différents âges et en particulier chez les prématurés.

1. L'auteur donne une courte revue de la littérature concernant l'activité enzymatique de l'anhydrase carbonique. Une importance spéciale a été accordée aux travaux cliniques les plus récents.

2. Il expose sa méthode qui est une modification de celle de Meldrum et Roughton.

3. Il donne un certain nombre de valeurs normales de l'activité de l'anhydrase carbonique du sang chez l'adulte, les enfants de différents âges et les nourrissons. On peut en conclure que l'activité de l'A.C. chez le nouveau-né est à peu près le tiers de celle de l'adulte et qu'elle augmente avec l'âge chez le nourrisons et chez l'enfant.

4. L'activité enzymatique du sang des prématurés n'est que d'environ 5 à 10 % de celle de l'adulte. Elle augmente lentement pendant les premiers mois de la vie.

5. L'auteur étudie les relations qui existent entre l'activité enzymatique et le degré de maturité, la taille et le poids des enfants à la naissance. Il semble y avoir une bonne corrélation entre l'activité enzymatique et le degré de maturité.

6. L'auteur étudie l'influence d'une faible activité enzymatique sur la cyanose et la vitalité des prématurés; il n'est pas en mesure de confirmer les résultats de Stevenson, d'après lesquels la cyanose est souvent associeé à une faible activité de l'anhydrase carbonique.

7. Il montre qu'il existe une proportionalité entre l'activité enzymatique et le taux du zinc sanguin.

Studien über Kohlensäureanhydraseaktivität bei Kindern. 1. Enzymaktivität im Blut von Säuglingen und Kindern verschiedenen Alters, besonders Frühgeborenen.

1. Es wird eine kurze Übersicht der Literatur über das Kohlensäureanhydraseenzym gegeben. Dabei wurde besonderes Gewicht auf frühere klinische Arbeiten gelegt.

2. Verfasser beschreibt seine Methode, welche eine Modifikation der Kahnmethode von Meldrum und Roughton ist.

3. Es werden eine Anzahl Normalwerte der Kohlensäureanhydraseaktivität im Blute Erwachsener, Kinder verschiedener Altersgruppen und Säuglingen angegeben. Es wird die Folgerung gezogen, dass diese Aktivität bei neugeborenen Kindern etwa ein Drittel der bei Erwachsenen beträgt, und dass die Enzymaktivität bei Kindern mit zunehmendem Alter steigt.

4. Es wird gezeigt, dass die Enzymaktivität im Blute frühgeborener Säuglinge nur etwa 5—10 % der bei Erwachsenen beträgt. Sie steigt langsam während der ersten Lebensmonate.

5. Die Beziehungen zwischen der Enzymaktivität und dem Grade der Reife, der Länge und dem Gewicht bei der Geburt werden diskutiert. Es scheint eine gute Korrelation zwischen Enzymaktivität und Reife zu bestehen.

6. Die klinische Bedeutung der niedrigen Enzymaktivität für Cyanose und Lebensfähigkeit bei frühgeborenen Kindern wird diskutiert. Verfasser war nicht imstande, Stevensons Erkenntnis zu bestätigen,

dass die Cyanose oft mit einer niedrigen Kohlensäureanhydraseaktivität vergesellschaftet ist.

7. Es wird Proportionalität zwischen der Enzymaktivität und dem Zinkgehalt des Blutes nachgewiesen.

Estudios sobre la actividad del enzima de la anhidrasa del ácido carbónico en el niño. Actividad enzimática en la sangre de niños de distintas edades y particularmente en prematuros.

1. Se da un breve resumen de la bibliografia sobre el enzima de la anhidrasa del ácido carbónico poniéndose especial interés en los primeros trabajos clínicos.

2. Se describe el método del autor que es una modificación del método de la barca ("boat method") de Meldrum y Roughton.

3. Se dan un número de valores normales sobre la actividad enzimática en la sangre de adultos, niños en grupos de distintas edades y lactantes. Se saca la consecuencia que la actividad de los recién nacidos es aproximadamente una tercera parte de la de los adultos y que la actividad enzimática en los niños aumenta con la edad.

4. La actividad enzimática en la sangre de niños prematuros es sobre un 5—10% de la de los adultos. La actividad aumenta despacio durante los primeros meses de la vida.

5. Se discuten las relaciones entre la actividad enzimática y el grado de prematuridad, longitud y peso de nacimiento. Parece existir una buena correlación entre actividad enzimática y madurez.

6. Se discute la importancia clínica de la baja actividad enzimática para la cianosis y vitalidad en niños prematuros. No le fué posible al autor confirmar el encuentro de Stevenson de que la cianosis va amenudo asociada a una baja actividad de la anhidrasa del ácido carbónico.

7. Se demuestra una proporción entre la actividad enzimática y el contenido de cinc en la sangre.

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FROM THE CAROLINE INSTITUTE'S PEDIATRIC CLINIC AT NORRTULL HOSPITAL (CHIEF: PROF. A. WALLGREN) AND THE PEDIATRIC CLINIC OF THE UNIVERSITY HOSPITAL, UPPSALA (CHIEF: PROF. B. VAHLQUIST).

Studies on Diphtheria

VI. The Effect of Diphtheria Immunization of Newborn Prematures

by

BO VAHLQUIST and FOLKE NORDBRING

In an earlier paper we have shown that, contrary to the general assumption, the antibody production in full-term newborns after immunization with diphtheria toxoid is fairly satisfactory, though the reaction is slower than normally (VAHLQUIST et al., 1948). Now we have taken up the question of how newborn prematures respond to a similar antigen action.

Material

The series comprised 15 children, with birth weights between 1,350 and 2,370 g. The vaccinations were done on the 1st to the 10th day of life. The vaccine was the same as that used for previous investigations (State Bacteriological Laboratory; 45 flocculation units per ml). Given subcutaneously in the thigh, the amount of vaccine varied between 0.2 and 1.0 ml, the larger doses being divided equally on either leg. To ensure full effect of the vaccination only such children were included as had an inherited antibody titre of less than 0.1 antibody units per ml. As usual, some of the vaccinations gave rise to infiltration but never to general reactions. All the children showed a satisfactory increase in weight on a standard diet of mother's milk reinforced with proteins.

As before, C. Jensen's technique was used for the antitoxin titrations.

Results

The results of the immunizations are given in table 1.

Table 1.

Antitoxin response following immunization against diphtheria in newborn prematures.

No.	Vaccine dose, flocculation units	Vaccination, day of life	Weight at birth, g	Antitoxin titre AU/ml			
				Before inoculation	After 4—5 weeks	After 12—18 weeks	After 26—40 weeks
1	9	5	1 350	< 0.005	0.0016	0.002	—
2	9	6	1 620	< 0.0004	< 0.0006	0.048	0.0011
3	9	5	2 130	< 0.007	< 0.0006	0.0009	—
4	22.5	1	1 480	< 0.0004	0.0035	—	0.002
5	22.5	2	1 830	< 0.0003	< 0.0003	—	0.002
6	22.5	7	1 910	< 0.0005	< 0.0005	—	< 0.0005
7	22.5	10	2 000	< 0.00045	< 0.0005	0.0138	—
8	45	2	1 360	< 0.0005	< 0.0005	0.0009	—
9	45	3	1 480	< 0.0005	0.0005	< 0.0004	—
10	45	4	2 000	0.0004	0.04	0.013	—
11	45	6	2 000	0.0004	0.003	0.014	—
12	45	6	2 050	< 0.0004	< 0.0006	—	—
13	45	4	2 070	< 0.0004	0.0008	0.003	—
14	45	9	2 095	< 0.0003	< 0.0003	—	0.0005
15	45	10	2 370	< 0.0005	—	0.02	—
				Median	< 0.0005	0.008	[0.0011]

Discussion

The amount of antigen administered in these experiments was very high, the median for the whole series being about 20 flocculation units per kg body weight. But this does not imply a corresponding increase in the effect of the antigen. In children of school age the production of antibodies seems actually to remain the same even if the amount of toxoid is raised above the usual 45 flocculation units (equivalent to about 1 flocculation unit per kg body weight).

The individual effect of the immunizations varied within wide limits. Five of the infants (Nos. 2, 7, 10, 11, 15) showed a fairly good response. No antibody response of any sort could be ascertained in two of the children. In the remainder the antibody

Table 2.

Antitoxin response following immunization against diphtheria in newborn prematures as compared with that obtained in a previous investigation (Vahlquist et al., 1948) in full-term infants of different age groups.

	n	After 4—5 weeks mean values	n	After 12—18 weeks mean values
Newborn prematures	14	< 0.0005[1]	10	0.008
Full-term newborns	15	< 0.0005[2]	13	0.02
2—3 mo. infants.....	15	< 0.02 > 0.01[3]	9	0.02
6—8 » »	15	0.02[3]	10	< 0.05 > 0.02

[1] 6 of 14 showed presence of diphtheria antibodies.
[2] 7 of 15 » » » » »
[3] 15 of 15 » » » » »

production was definite, but poor and often delayed. The results permit no conclusions as to whether a higher weight at birth is accompanied by a better antibody response.

The results here presented and our previous results of vaccination experiments with full-term children of various ages are compared in table 2. It will be seen that premature newborns not only react slow as the full-term ones do, but, in addition, the final titres are lower in the former than in the latter. It would seem reasonable to associate the premature child's greater susceptibility to infections and propensity to develop septic conditions with its slow and often poor response to bacterial antigens.

The investigation was supported by a grant from the State Medical Research Council.

Miss F. Wagenberg rendered valuable assistance with the diphtheria antitoxin titrations.

Summary

Fifteen prematures were inoculated with single doses of diphtheria toxoid between the 1st and 10th day of life. After four to five weeks only six of fourteen children showed a weak reaction, after twelve weeks

twelve of fourteen children had responded, but the median titre was only 0.008 antitoxin units/ml. The effect was weaker than that previously obtained by vaccination of full-term children.

Études sur la diphtérie. VI. Effet produit par l'immunisation antidiphtérique chez le nouveau-né prématuré.

15 prématurés furent innoculés avec une dose unique de toxoide diphterique entre le 1 et le 10 jour de la vie. Après un laps de temps variant de 4 à 5 semaines 6 seulement de ceux-ci parmi 14 présentaient une faible réaction. Mais après 12 semaines 12 sur 14 réagissaient, cependant le titre moyen d'antitoxine était seulement de 0.008 unité par cc. Cette réaction s'est montrée plus faible que celle que nous avons déjà obtenue en vaccinant des enfants nés à terme.

Studien über die Diphtherie. VI. Die Auswirkung von Immunisierung gegen Diphtherie bei Frühgeborenen.

Fünfzehn Frühgeborene wurden mit Einzeldosen von Diphtherietoxin zwischen dem 1sten und 10ten Lebenstag geimpft. Nach vier bis fünf Wochen wiesen nur sechs aus vierzehn Kindern eine schwache Reaktion auf, nach zwölf Wochen hatten zwölf aus vierzehn Kindern reagiert, aber der Titermittelwert betrug nur 0,008 Antitoxineinheiten pro ml. Die Wirkung war schwächer als jene gewesen, welche man früher bei der Impfung von rechtzeitig geborenen Kindern erhalten hatte.

Estudios sobre difteria. VI. Efectos de la inmunización antidiftérica en los prematuros recien nacidos.

Quince prematuros fueron inoculados con una dosis aislada de toxoide diftérico entre el primero y décimo día de la vida. Después de cuatro a cinco semanas solo seis de catorce niños mostraron una débil reacción, después de doce semanas doce de catorce niños habían respondido, pero el título medio era solo de 0,008 unidades de antitoxina por ml. El efecto fué más débil que el obtenido anteriormente vacunando a niños nacidos a término.

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Tuberculin Hemagglutination Studies in Active Tuberculous Infections, Benign and Virulent[1]

by

J. C. PETERSON, R. LAGERCRANTZ, S. I. ROLLOF and J. LIND

Introduction

The development of the Middlebrook-Dubos hemagglutination test for humoral antibody in tuberculous infections resulted from the evolution of a new technique. A few years ago it was demonstrated that one could use red blood cells which had been coated with virus particles for a specific agglutination test for that particular virus, etc. Such a test had fairly wide use in the study of influenza and has proved to be a useful adjunct to the study of this infection. In 1948 MIDDLEBROOK and DUBOS modified this technique for use in the study of tuberculous infections. They developed a preparation of Old Tuberculin which would satisfactorily sensitize red blood cells and showed that animals with tuberculous infection and people with tuberculosis had agglutinins which could be demonstrated with this indicator. The test has since then been modified in minor detail by a number of investigators and a commercial firm[2] has made available to investigators a supply of tuberculin satisfactory for the test.

A number of studies, beside those of the original authors, have

[1] These studies have been supported by a grant from Svenska Nationalföreningen mot Tuberkulos.

[2] Lederle Laboratories, Pearl River, New York, who kindly supplied the special quadruple strength Old Tuberculin used in this study.

been carried out to examine the specificity and reliability of the test. These studies have demonstrated that normal individuals who are tuberculin negative do not have antibody demonstrable by this technique. The studies have also shown that heterologous infections do not excite the development, *de novo*, of antibody which will affect the test. It has been shown that the great majority of individuals who have tuberculous infection which can be demonstrated by the recovery of the specific mycobacterium do have antibody which can be demonstrated by the technique.

The hopes which some of the early workers had for the test, namely that it would be a certain method for the differentiation of tuberculin allergy and active tuberculous infection, and that it would be a reliable guide to the patient's reaction to his infection, do not at this time seem to be justified. While most of the cases of active tuberculosis do have the antibody, the amount is quite variable and it has not been possible to correlate the amount present with the activity of the infection, except that there has been a suggestion that those who are in terminal stages with overwhelming infection do not have antibody (2). Some observers have reported that those with long arrested infections also have little or no antibody, but this feature has apparently varied with different observers.

In experimental infections, with virulent organisms it is always possible to demonstrate the development of the antibody in variable amounts. This has also been true with regard to experimental infections in animals using B. C. G. as the infecting agent. There it has been possible to show the development of relatively high titers in all of the animals which were given fairly large amounts of B. C. G. (3). In the routine vaccinations of humans with standard amounts of B. C. G. (2—3) it has not been possible to show the development of antibody with any degree of consistency which would make the test a valuable adjunct to the tuberculin test as a means of determining the presence of immunity.

In fact, at this time it is not clear just what significance can be attached to the presence of antibody following vaccination or in infected individuals. From the information at hand it would

seem that a negative test, in an individual who is not in a state of extremis, and where the observation has been repeated several times over the course of 3—5 months, probably indicates the absence of a tuberculous infection. *A priori* reasoning would suggest that the failure to show an increasing titer of antibody in an individual who had been previously vaccinated against tuberculosis with B. C. G. would also be indicative of the absence of an active tuberculous infection. On the other hand, strong and increasing titers arising without the presence of any known stimulant, such as tuberculin tests, repeated vaccinations etc., would surely suggest the presence of a concomitant tuberculous infection.

Whether the quantitative aspects of the hemagglutination tests are of more value than similar aspects of tuberculin testing is not known, but it would seem reasonable to expect that it might have more significance in that the tuberculin test is purely allergic in nature, dependent for the strength of the reaction more on the constitutional factors of the individual than upon its relationship to the specific immunity, while the hemagglutination test does at least measure the patient's ability to develop a specific antibody against the etiological agent of his infection, whether or not the antibody is of significance with regard to his immunity.

The technique of the test and its modifications has been described several times in the literature (1—4). Here we will need only a brief outline of the procedure. The antibody present in the patient's serum is titrated in the same manner that it is in any agglutination test, by trying serial dilutions of the serum against a measured and constant amount of the indicating antigen. The antigen in this case is the red blood cell of the sheep, coated with a standardized amount of Old Tuberculin, specifically prepared for the purpose and free from any undesirable effect upon the cell. The serum to be tested must have the heterophile antibody, which might be present, removed by absorption before the test. This is done with washed and packed plain sheep red blood cells. It would be possible to use O human cells as an indicator in the test if a good supply of these is at hand and this would obviate the necessity for the preliminary absorption of the serum to get rid of the heterophile antibody. The technique which we have used has been modified from that described by SMITH and SCOTT (4), the only modification being the slight alteration in the dilution of serum used, 1 to 4 instead of 1 to 2, and the use of slightly smaller amounts of the packed cells for ab-

sorption, 0.2 ml twice. Each serum is controlled to be certain that there is no non-specific agglutinin present by setting up the strongest dilution used against plain sheep red cells.

We have performed about 600 tests, some few of which have represented duplicates which were run to examine the technique from the viewpoint of its reproducibility. We can say that the tests can be repeated with identical results when the routine of the test is carried out meticulously though there is some tendency for the sera to lose some antibody with storage over a period of several weeks.

The reading of the tests usually does not pose any problem. The agglutinated cells are adhesive enough that the clumps are not readily broken up with shaking, making it easy to differentiate packing and true agglutination. The reaction is not fully developed immediately after the incubation in the warm water bath but must stand overnight at room temperature. It is a good practice to read each test twice, once as the cells are resuspended and again after about 45 minutes. The second reading, based upon the speed with which the agglutinated cells fall out of suspension and the type of cluster which they form in the bottom of the tube is a very good check on the first reading, often giving the technician aid in deciding any doubt as to the exact titer of the reaction. The level of positivity is taken as that at which the cells are quite completely agglutinated, and the doubtful or partial agglutinations are not included except where this occurs in the first tube, then if there seems to be fairly definite though not complete agglutination the test is recorded as showing an agglutination of less than 1—4. It is quite certain that these tubes would show definite agglutination if larger amounts of serum could be used in the test. Also in some instances we have interpreted a parted agglutination at 1—16 as positive, 1—8, a dilution not ordinarily run in our test. The particular dilution scheme which we selected was based on the fact that there is occasionally some difficulty in getting more serum from the new born babies and does not indicate that we lack confidence in the results of tests run at higher serum concentrations.

Observations

In this study we are trying to find out what can be learned about the progress of tuberculous infection and the development of the specific immunity in tuberculous infections from the tuberculin hemagglutination test. We have been able to make observations in a variety of differing categories, but at this time we want to discuss only the findings in cases of primary tuberculosis and of vaccination with B. C. G. in infants and children.

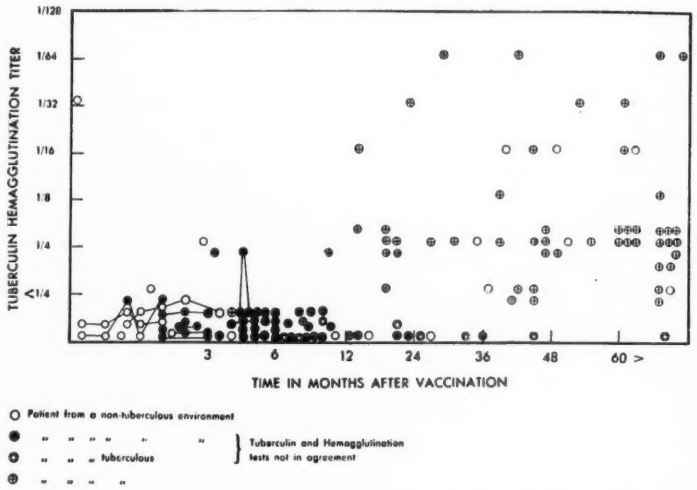

Fig. 1. Hemagglutination titers in the infants vaccinated in the neonatal period.

B. C. G. vaccinated

First we wish to consider the tests done at various times after the vaccination on sera from infants and young children who had been B. C. G. vaccinated at birth (within the first week of life). The results of these tests are shown in figure 1. The legend indicates several variables within the group, those with possible secondary exposure and where the tuberculin tests and hemagglutinations were inharmonious.

From this chart it is quite apparent that infants do not respond, within the first year after B. C. G. vaccination with the production of any measurable amounts of agglutinins. The exceptions to this, four out of sixty-four tests, are all at the lowest levels and one had had several tuberculin tests while another had a possible exposure to infection at home. On the other hand those tests which were run after the first year, on infants who had been vaccinated at birth, show low to moderate levels of agglutinins in all but twelve out of sixty-eight tests, an almost exact reversal

of the first observation. This must mean that the antibody is formed very slowly after the vaccination and that the stimulus from the vaccination extends over a period of much more than a year, or that the antibody observed in these individuals is the result of superinfection. The group as a whole is not representative of a normal situation, as the tests which were performed, after the first few months, on infants and young children vaccinated at birth were almost all from homes where there had been tuberculosis; this tuberculosis was, of course, the reason of why they were being brought back for the supplementary observations. (Children under 6 years of age, from the non-tuberculous homes are not followed with yearly checks to see that they maintain their tuberculin sensitivity.) It must also be recognized that almost all of these infants and young children had received a tuberculin patch test, or ointment test, three days before the serum for the test was obtained. This may then represent an anamnestic response, stimulated by the tuberculin test rather than a response to superinfection. Against this hypothesis is the fact that three days is a very short time in which to secure such a response and the fact that the infants less than a year of age had, in many instances, also been the recipients of tuberculin tests in exactly the same manner. This point needs further study as will become more apparent from observations in older groups, to be discussed next.

In figure 2 are shown the results of tests done on children who were past the neonatal period when they were first given B. C. G. vaccine. The variables within the group are explained in the legend. In relation to the question, as to whether or not the tuberculin test alone can bring about the development of an anamnestic response within three days, it is interesting to observe that in children who had first received an ointment test and then an intradermal tuberculin, and who were tested as controls, had in 14 out of 19 tests some hemagglutinins before they were given the B. C. G. vaccine, though in three this was only a trace of antibody. Presumably, in these tuberculin negative individuals, this antibody response came within a period of six days, a time interval which usually is not great enough for the development

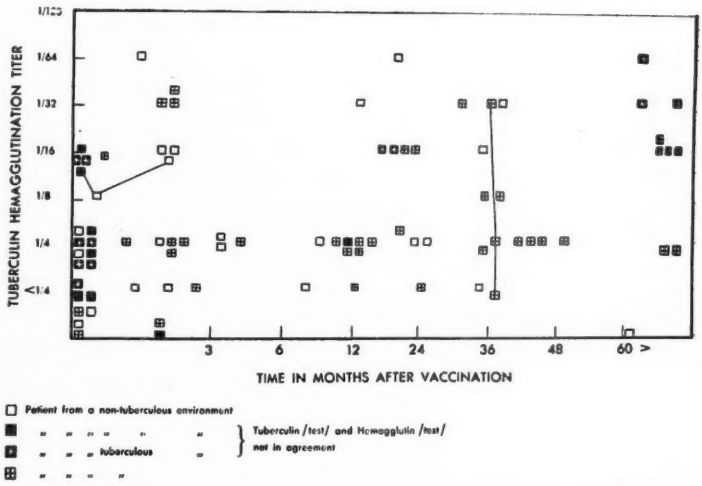

Fig. 2. Hemagglutination titers in the infants and children vaccinated in early childhood.

of measurable antibody. It is also worthy of note that seven out of the eleven, who showed these prevaccination levels of antibody, were from homes where there was some tuberculous infection and in some instances there was a possibility of recent exposure, the reason why they were coming in for vaccination. The numbers are too few to justify individual analyses of these various factors. In children in this group, in contrast with the infants vaccinated in the neonatal period, there was a prompt production of hemagglutinins and all but two of twenty had some antibody, three at the level of less than 1—4 but eight had titers of 1—16 or greater. It should be pointed out that seven of these responders came from homes with tuberculous infection, but this was also true of one of the two who failed to respond. In the time intervals beyond six months there were only three out of forty tests which did not show some degree of antibody, in some instances fairly high levels three to six years after the primary vaccination, again mostly in children from homes with some record of tuberculosis. In this group we found, in several instances, fairly high levels of antibody

in individuals who had lost their tuberculin sensitivity, at least as far as that can be judged from the salve test.

In one of the individuals of this age group, we were able to make several observations shortly after an infection which was at first suspected of being tuberculosis, but which was probably an acute infectious mononucleosis. The child had a fairly high titer of antibody on the first observation, probably a non-specific augmentation, which promptly fell over a period of six weeks to a bare trace. In this case the tests were of some real value in differentiating the nature of the infection as she had large mediastinal lymphnodes with some pulmonary atelectasis. The diagnosis of probable infectious mononucleosis was made on the basis of a rising heterophile antibody titer, also she was barely sensitive to 1.0 mg O.T. intradermally.

It should again be pointed out that the majority of these tests were carried out after the children had received a tuberculin test three days before. In our preliminary thinking of this question we had thought that this period would be too short to find interference from an anamnestic response.

Primary tuberculosis

We have also been able to make 175 titrations on children of various ages, at various times after the onset of primary tuberculosis. A considerable number of these tests were serial tests on patients under treatment in the Norrtull Hospital and some of these of special interest will be considered individually. In one or two instances these individuals had had prior B. C. G. vaccination but as they did not show anything of particular significance from the point of view of the tests they will not be considered separately. In most instances it was not possible to determine the exact time at which the individual received his infection with virulent tubercle bacilli so we have chosen to make the zero point on this chart begin with the onset of symptoms attributable to the tuberculous infection. This of course means that there was an additional time interval of some six to eight weeks, or more, for a record, as compared with that for B. C. G. vaccination, in which

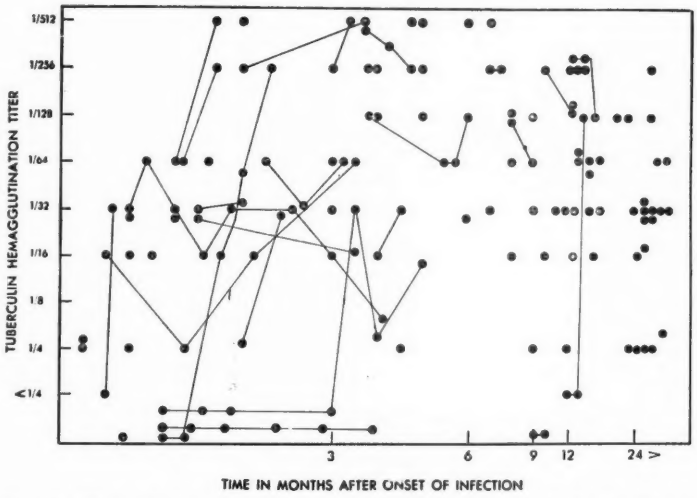

Fig. 3. Hemagglutination titers among individuals with clinically significant primary tuberculosis.

the individual could have been starting to elaborate antibody. It is unfortunate that it is very rarely possible to secure observations before the onset of symptoms or to know the exact time of infection.

The children with primary tuberculosis have been divided into two groups, those with clinically manifest primary tuberculosis and those with primary tuberculosis which was found quite fortuitously, and in whom the time of their exposure could be fixed with fair accuracy because they were being closely followed, after a known exposure or for other reasons. The test results of the first group are shown in figure 3, and those of the second group are shown in figure 4. This division was made in order to observe whether or not the severity of the process has any real significance as a modifying factor in the development of the hemagglutinins.

In generalization it can be said that the great majority of individuals, in the age group represented, develop detectable amounts of antibody within a few weeks to three months after

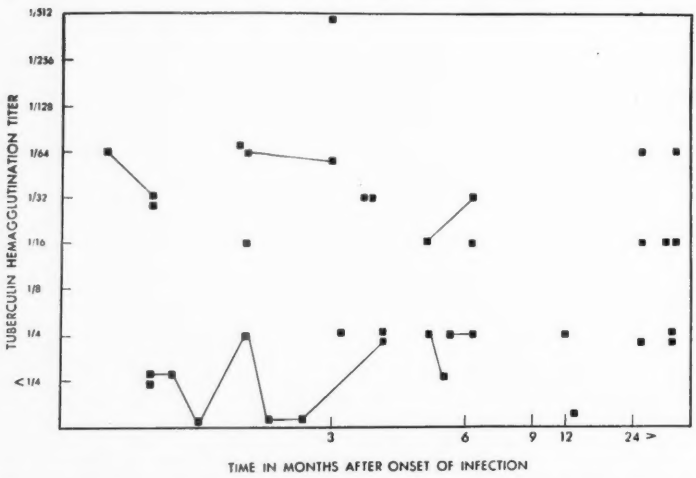

Fig. 4. Hemagglutination titers among individuals with minimal clinically inapparent, primary tuberculosis.

the onset of the clinical symptoms; the exceptions will be discussed individually below. The apex of the antibody response is usually achieved in the period of two to six months after the onset of symptoms and then gradually falls off to slightly lower levels during the next one to two years, though some had fairly high levels at a time many years after their infection has become latent or even healed. Only two individuals, in three tests, failed to show some antibody in the time period beyond three months. It should be said that the group did not include any individuals with progressive hopeless infections.

It would appear from a cursory examination of figures 3 and 4 that there is some tendency for the levels of hemagglutinins to be higher and be maintained high for longer period in those who had clinically active infections than in the group who had clinically inapparent infections, but the numbers are quite small and the statistical significance of the observation has not yet been determined.

In Fig. 5 are shown several curves representing serial observations on the same patient; the titers of the individual tests are

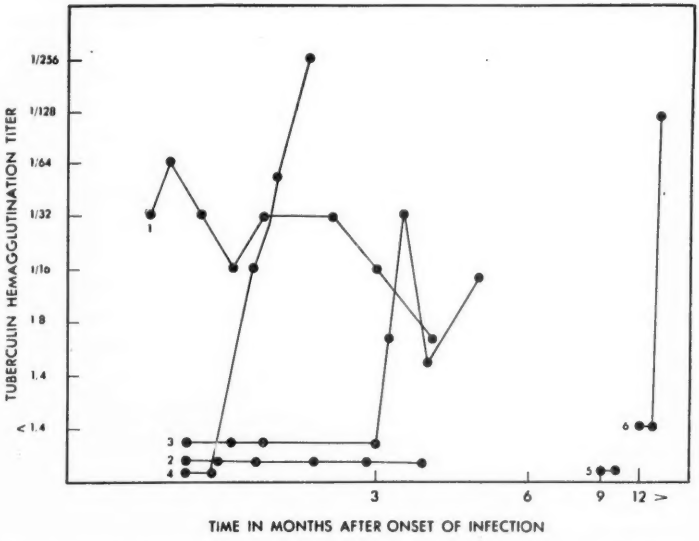

Fig. 5. Serial hemagglutination titers observed in selected cases of primary and post-primary tuberculosis.

connected by lines. These are worthy of individual comment: The curve (1) is from a girl who developed erythema nodosum about six weeks after her father was found to have pulmonary tuberculosis with cavitation. Her antibody level had risen to 1—32, three weeks after the onset of her symptoms and remained at that level with fluctuations of one degree up or down for four months. She had a very moderate pulmonary lesion and no bacilli could be demonstrated in her gastric washings. Curve (2) is that of the first patient's brother. He is said to have had a positive tuberculin test two years before without any significant clinical symptoms (in reality, therefore, his tuberculosis is probably not a primary infection but a post-primary infection). Shortly after his sister became ill he developed signs of a respiratory infection, with cough and fever. He was found to have a very minimal pulmonary lesion by roentgen examination but on two successive days he had tubercle bacilli in his gastric washings. Throughout

the first four months after the onset of this superinfection he has never showed any antibody response. Clinically he was essentially free from fever, never as high as 38° and his ESR did not exceed 18 mm except on one occasion four weeks after admission. Curve (3) is from an 11 years old girl from a family without tuberculous infection and the child had had no known exposure. She was sent home from school with cough and fever. She was found to have a positive tuberculin test and an ESR which remained for several months at a level of 42 to 50. In the hospital (five days after the first symptoms) she was fever free and did not seem ill. Her gastric washings were positive for human type tubercle bacilli and roentgen examination showed a minimary pulmonary infiltrate without any significant adenitis. The girl showed no antibody response for three months and then an abrupt rise which was not sustained but fell back to a lower level after a few weeks. Her tuberculosis was such a minimal infection that it probably would not have been recognized except for the practice of doing tuberculin tests after all febrile illnesses. Curve (4) is from a patient with miliary tuberculosis without meningitis. He was started on treatment with streptomycin and PAS[1] two weeks before our first observation. In spite of this relatively early treatment he promptly went ahead to develop a high level of antibody. Curve (5) represents a pair of observations on a child who had had a minimal but bacillus proven infection nine months before. Curve (6) represents a child who had a minimal infection a year before, and four weeks before our first observation he had an extension of his primary infection, or a superinfection.

These individual observations, some in cases with absolute proof of infection, would seem to lend some support to the thesis that those with well marked infection are more likely to develop higher levels of antibody, at least in primary infections. On the other hand a minimal superinfection may be contained by the patient without any significant antibody response, as is illustrated by curve (2).

It is apparent that single tests early in the course of the

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[1] Para amino salicylic acid.

infection may not show the presence of these hemagglutinins or they may be at levels so low that they could come from previous B. C. G. vaccination. As in all serological tests where the individual may have a low titer from some antecedant infection it is necessary to make serial tests, sometimes over a period of several months, before one can make an accurate and intelligent interpretation of the test. This is illustrated by the findings in curve 3 of figure 5.

Discussion

From the observations made in this study and from what has been previously reported there are several points which may be brought up for discussion.

Of greatest interest are the sustained and relatively high titers of antibody seen in the infants and children one or more years after their primary vaccination with B. C. G., whether the vaccination was done in the neonatal period or at a later time. Judging from the fact that the infants vaccinated in the neonatal period did not show any significant elaboration of antibody in the post vaccination period of three to twelve months, when they were from non-tuberculous environments or had been kept away from their tuberculous homes it would seem reasonable to assume that the primary B. C. G. vaccination is an inadequate stimulus for the development of these hemagglutinating antibodies in the very young infant. Then the finding that they have almost always elaborated such antibody beyond the period of the first year, when they were placed in homes with some theoretically possible chance for superinfection, must mean that they have actually had a superinfection which has been able to produce an anamnestic response though it has not been responsible for the production of a clinically significant infection. This hypothesis seems more attractive to us than the alternative that the B. C. G. infection remains active for these long periods of time and is capable of stimulating the elaboration of antibody at such a late date.

The validity of this observation needs to be tested under circumstances which will admit exact control of all the variables

and such a study is one which will be of more than simple biologic academic interest.

Another point with respect to the thesis that the antibody response in this group being due to superinfection is the apparent observation that the individuals in question were protected against virulent infection at least against its production of a clinical reaction even though they did not respond to the primary vaccination with the production of antibody. This may be interpreted in two ways. First, that the hemagglutinating antibody has no significance with respect to the immunity which follows B. C. G. vaccination and that it is just another unrelated but sometimes associated phenomenon like the allergy to tuberculin. Or, secondly, that in the young individuals the basic immunity was established, although at a level lower than would be associated with the evolution of high titers of antibody. This would of course bring up the question as to whether or not humoral antibody ever plays any role in the picture of immunity to tuberculosis. In the past many infections have been thought, as tuberculosis is now regarded, to engender only tissue immunity. This was true of several of the virus infections which we now know to be completely preventable, in their ordinary development, by the passive infusion of antibody. While it is quite possible that the antibody involved in the hemagglutination test is not related to tuberculosis immunity these observations seem to us to indicate the need for restudy of the question as to whether humoral immunity is of no significance in tuberculosis.

If it is true that the phenomenon is the result of superinfection this may be looked upon as significant proof of the value of B. C. G. vaccination in the prevention of clinical response to tuberculous superinfection in infants and these studies reveal that it occurs with unexpected frequency. This would also indicate that homes where there are persons who have had tuberculosis are of potential danger, at least for an infant, even when the infection of the person in the home is thought to be clinically non-infectious. A young child living in such a home is a better, or more sensitive, indicator of the infectiousness of the patient with a relatively inactive or latent infection than are the usual laboratory studies.

One other point in favor of the hypothesis that superinfection and not prolonged action of B. C. G. is the explanation for this antibody is the fact that reversal of the tuberculin test does not occur in B. C. G. infections at this late period. If the infection was active enough to produce antibody at such a late date it should also occasionally bring about a late reversal of the tuberculin test to positive, a reaction which is not generally expected by those in the B. C. G.-vaccination field.

These observations would seem to indicate that the use of the hemagglutination technique has a field of usefulness in the study of the dynamics of tuberculous infection and in the development of some aspects of tuberculous immunity. It should adjunct in the diagnosis of superinfection, in those who have been vaccinated with B. C. G., or in individuals who were in the quiescent or healed period of primary infection with virulent bacilli, in other words in those who have a true or suspect infection but who were known to have been, or were supposedly, tuberculin positive before the onset of the infection in question.

Summary

1. We have presented in a preliminary form the results of observations on the tuberculin hemagglutination test in relation to the dynamic development of the represented antibody in infants vaccinated in the neonatal period and in infants and children vaccinated at a period of time remote to the neonatal period.

2. We have also examined the dynamic development of the hemagglutinating antibody in patients with primary tuberculosis, and have made some preliminary observations as to the relationship of the amount of antibody formed and the severity of the primary infection.

3. Some aspects of the significance of these observations have been discussed, particularly the failure of infants to develop antibody during the first year after B. C. G. vaccination and the subsequent appearance of antibody among those infants when they reside in homes with a history of tuberculous infection.

Etudes de l'hémoagglutination au cours des infections tuberculeuses aigues, bénignes, virulentes.

1. Nous avons présenté sous forme préliminaire les résultats d'observation concernant le test d'hémoagglutination à la tuberculine en relation avec le développement dynamique des anticorps chez des enfants vaccinés en période néonatale et chez d'autres vaccinés à une phase éloignée de cette période.

2. Nous avons également étudié le développement dynamique des anticorps donnant le phénomène de l'hémoagglutination chez des malades atteints de tuberculose de primo-infection et nous avons donné quelques idées préliminaires concernant la relation existant entre la quantité d'anticorps formée et la sévérité de cette primo-infection.

3. Nous avons discuté la signification de ces observations particulièrement le pouvoir négatif qu'ont les enfants de développer des anticorps pendant la première année qui suit la vaccination par le B. C. G. et leur apparition ultérieure lorsqu'ils habitent dans un milieu où existe un foyer d'infection tuberculeuse.

Beiträge zur Tuberkulinhemagglutination bei gutartigen und bösartigen aktiven tuberkulösen Prozessen.

1. Wir haben in einleitender Form die Beobachtungsergebnisse über die Tuberkulinhemagglutinationsprobe mitgeteilt in bezug auf die dynamische Entwicklung des besprochenen Antikörpers sowohl bei Säuglingen, welche im Zeitraum kurz nach der Geburt geimpft, als auch bei Säuglingen und Kindern, die lang nach der Geburt geimpft worden waren.

2. Wir haben auch die dynamische Entwicklung des hemagglutinierenden Antikörpers bei Patienten mit Primärtuberkulose geprüft und einige präliminäre Beobachtungen über den Zusammenhang zwischen Menge des gebildeten Antikörpers und Schwere der Erkrankung gemacht.

3. Es wurde die wesentliche Bedeutung dieser Beobachtungen besprochen, insbesondere, dass bei Säuglingen im 1. Jahr nach der BCG-Impfung kein Antikörper gebildet wird und dass bei diesen Säuglingen Antikörper erscheint sobald sie sich in Heimen aufhalten, wo eine tuberkulöse Infektion zu verzeichnen war.

Estudios sobre la hemaglutinación tuberculínica en las infecciones tuberculosas activas benignas y virulentas.

1. Se presentan en forma preliminar los resultados de la prueba de hemaglutinación tuberculínica en relación con el desarrollo dinámico de los anticuerpos representados en niños vacunados en el período neonatal y en lactantes y niños vacunados en períodos posteriores de la vida.

2. Tambien se han examinado el desarrollo dinámico de los anticuerpos hemaglutinantes en pacientes con tuberculosis primarias y se han efectuado algunas observaciones preliminares de la relación entre las cantidades de anticuerpos formados y la severidad de la primo-infección.

3. Se discute la significación de algunos aspectos de estas observaciones, particularmente la incapacidad de los niños para desarrollar estos anticuerpos durante el año siguiente a la vacunación B. C. G. y la subsiguiente aparición de anticuerpos entre estos niños cuando residen en medios con historia de infecciones tuberculosas.

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FROM THE SAMARITEN CHILDREN'S HOSPITAL, STOCKHOLM.
(CHIEF: PROFESSOR NILS MALMBERG.)

Retrolental Fibroplasia in Sweden

by

INGEMAR BJELKHAGEN

HEDLUND-KRISTIANSEN reported the first case of retrolental fibroplasia in Sweden in April 1948, the diagnosis being established by HENNING MAGNUSSON.

Case 1. M. J. Girl, born 9/9 1947. First child. Mother's age 18, unmarried, healthy during pregnancy apart from slight uterine bleeding for a day or two at the beginning. Parturition normal, 3 months before term. Weight at birth 1 100 g, lowest weight 950 g. Treated at the Samariten Children's Hospital for 4 months, 6 weeks of this time being spent in an Aga couveuse. Not breast fed, but given breast milk for 4 weeks and after that an addition of citric acid whole milk. Extra amino acids (Aminosol glucose), vitamin C and later on iron were given. Hemoglobin, lowest 67 per cent. Prothrombin index, lowest 30 (vitamin K was given). A total amount of 500 000 I.U. of vitamin D_2 (Fortedol) was administered. Penicillin was given prophylactically for one week. At the age of 4—5 months the mother noticed that the infant did not fix her gaze. At the age of 6 months, 15/3 1948, the eyes were examined: anterior chamber very shallow, the lens clear, behind this, in the case of falling light, a greyish green reflex with fine, delicate vessels. Double-sided changes (Lindblad). W.R. and x-ray of skull negative. An electroretinogram was taken 6/4 1948: the retina probably not functioning (Karpe). Toxoplasmosis test taken on mother and child negative. The infant showed an increasing aversion from light and often rubbed her eyes. The eyes became more and more hollow and shrunken, particularly the right one, with low tension. At the age of 3 1/2 the patient has not learnt to walk and is imbecile. She has a predilection for sitting and rubbing her eyes; at the same time she rocks herself backwards and forwards (see pictures). Her legs are spastic. Nothing special with regard to internal organs. No hemangiomas.

Case 1.

Another 2 cases of fibroplasia have as premature infants been treated at "Samariten". Both mothers and one infant had also positive toxoplasmosis serological reactions, no blood test having been taken on the other infant. One of the infants is described as a case of toxoplasmosis by GRANSTRÖM and MAGNUSSON (case 2).

Case 2. B. H. Boy, born 28/11 1945. Youngest of 3. Mother's age 36, married, in good health during pregnancy apart from slight uterine bleeding at the end. Parturition normal, 2 months before term. Weight at birth 1 160 g, lowest weight 960 g. Treated at "Samariten" for 3 1/2 months, kept in an Aga couveuse for 7 1/2 weeks. Not breast fed, but given breast milk + citric acid whole milk. Hemoglobin, lowest 70 per cent. Prothrombin index, lowest 25. Vitamin K 40 mg. In addition Aminosol glucose, vitamin C, iron and a total amount of Fortedol of 500 000 I.U. were given. At 5—6 months he was unable to fix his gaze and he also had a grey pupil. He was constantly rubbing his eyes, especially the left one. An eye-specialist was not consulted until the boy was 14 months old, when it was then ascertained that the anterior chamber was almost entirely occluded, that the right cornea was considerably blurred, that the left was almost clear, that the lenses and the irises bulged, that synechiae were particularly developed in the left eye and that there was no red reflex, that it was partly opaque on the left and entirely so on the right (Granström). In April 1947 the boy was treated at the Sachs' Children's Hospital. Toxoplasmosis tests taken on mother and child positive. X-ray of skull negative. Internal organs nothing noteworthy. Imbecile. No hemangiomas.

Case 3. C. Z. Boy, born 18/1 1946. An only child. Mother's age 23, divorced, gonorrhea during pregnancy, otherwise healthy. Parturition normal, 3 weeks before term. Weight at birth 1 390 g, lowest weight 1 240 g. Treated for nearly 4 months at "Samariten". Kept in an Aga couveuse for 1 month. Not breast fed. Breast milk + citric acid whole milk, amino acids, vitamin C, iron and Fortedol, 500 000 I.U. were given. Hemoglobin, lowest 70 per cent. Prothrombin index, lowest 40, vitamin K 15 mg. At the age of 5 months it was discovered that his sight was bad and that there was something white in the pupil. He was examined by an eye-specialist at Västerås' General Hospital in April 1948 and January 1949. The left eye was then found to be considerably smaller than the right. There was a grey formation with fine, delicate vessels behind the lens on the left. He was examined at "Samariten" on 20/3 1951. An atrophic iris was then discovered on the left eye, the anterior chamber was shallow, there were synechiae, nystagmus and a grey sheath behind the lens. In the right eye there was a retinal fold across the papilla on the fundus, where chorioiditic centres were found.

There was no retrolental membrane in this eye (Lindblad). The patient can walk about without knocking against anything, at least in places with which he is familiar. On the right of the forehead there is a distinct superficial network of veins. Internal organs nothing noteworthy. No hemangiomas. Mentally normal. The mother's toxoplasmosis test was positive, but the patient's was not taken.

Apart from these three cases, there have been three others that have been under observation for retrolental fibroplasia at the Samariten Children's Hospital.

Case 4. I. K. Girl, born 23/12 1947. An only child. The mother's age 24, married, healthy during pregnancy, no uterine bleeding. Parturition normal, 6 weeks before term. Weight at birth 1 830 g, lowest weight 1 640 g. Treated for one month at the Sachs' Children's Hospital. No couveuse. Breast milk for 3 months. Aminosol glucose. Prothrombin: no coagulation. Prothrombin index normal after the administration of vitamin K. At the age of 5—6 months the mother noticed that the infant had a squint in her left eye and was not able to fix her gaze. Various hospitals were visited, toxoplasmosis test was taken in August 1948, which proved negative. In December 1948 the author referred the child to "Samariten" for suspected retrolental fibroplasia, which was then confirmed. The left eye was moderately microphthalmic. The anterior chamber was extremely shallow. The lens was clear, with grey tissue all along the back. Nystagmus. Right eye normal apart from nystagmus (Lindblad). Glaucoma in left eye in September 1949, but it subsided without operation. While at the Sachs' Children's Hospital in October 1949 an encephalogram was taken, which was normal. EEG also normal (Melin). Admitted to "Samariten" in February 1951 for control. The left eye mainly unchanged. Right eye: papilla was somewhat pale and blurred, nasally there was protrusion of vessels, temporally the vessels were small (Lindblad). Internal organs, nothing worthy of note. No hemangiomas. Mentally normal.

Case 5. B. S. Boy, born 17/11 1947. Younger of 2. The mother's age 37, married, in good health during pregnancy, no uterine bleeding. Parturition normal, but 2 months before term. Weight at birth 1 580 g. Treated for 2 months at the Södertälje General Hospital, in an Aga couveuse for 3 weeks. Breast milk + 1/2 gruel. Vitamin D_2 500 000 I.U. Just after discharge from the hospital the mother noticed that the boy did not fix his gaze. He was examined at several places, inter alia the Caroline Hospital in November 1948, where it was discovered that the eyes were smaller than normal, that the pupils did not react to light and that there was a membrane with vessels on the surface behind the lens. On an occasion when his sister visited the out-patients' department at

Case 5.

"Samariten" in the spring of 1950 the author heard about the patient's eye-affection, and from the symptoms suspected fibroplasia. This diagnosis was confirmed after an examination in June 1950. In the left eye there was typical fibroplasia with a shallow anterior chamber, a clear lens and a greyish white membrane retrolentally. In the right eye there was cloudiness, maximal in the centre, the anterior chamber being extremely shallow or entirely occluded. Both eye-balls sunken. Toxoplasmosis tests taken on mother and child were negative. He has been controlled several times at "Samariten". He is extremely sensitive to light and often rubs his eyes. He can probably distinguish objects to a certain extent in very bright light. The condition of the eye has remained largely unchanged since 1950, according to the last examination made in February 1951. Right eye: the cornea clouded for the most part, probably adherent to the iris. Look in eye lacking. Tension raised. Left eye: nystagmus, cornea clear, anterior chamber shallow, pupil average width, did not react to light, lens clear, behind the surface of the lens grey membrane probably with a vessel (Lindblad) (see picture). X-ray of skull negative. Internal organs, nothing worthy of note. No hemangiomas. Mentally normal.

Case 6. I. S. Girl, born 11/1 1950. An only child. Mothers age 22, married, healthy during pregnancy, no uterine bleeding. Parturition normal, 2 1/2 months before term. Weight at birth 1 360 g, the lowest 1 130 g. Treated at the Södertälje General Hospital for nearly 2 months, and kept in an Aga couveuse for the first part of this time. Breast fed for 4 months, additional quantity for 2 months. No vitamin D_2. At the age of 3—4 months the infant began to squint, and at 5 months it was noticed that the left eye was smaller than the right. The patient was treated at the Eye Clinic of the Caroline Hospital from 26/1—8/2 1951 for Fibroplasia retrolentalis + Retinochorioiditis oc. amb. W.R. negative. X-ray of skull and orbitae showed nothing worthy of note. Toxoplasmosis test negative. The right eye somewhat reduced in size, behind the lens a grey diffuse mass. Squinted inwards approximately 20°. The left eye followed the source of light. Cornea and lens showed nothing worthy of note, pigment of the retina poor, occasional red spots, probably bleedings. A large retinal fold, and a light spot with dark lumps of pigment and exudate looking like active centre of retinochorioiditis. Downwards temporally behind the lens white cloudiness preventing any study behind this part of the fundus. The papilla and vessels showed nothing worthy of note. After being discharged she was treated with a series of Jodbismol (iodine and bismuth) intramuscular injections. The mother thought that they did good, though objectively there was no improvement. In April 1951 the girl visited "Samariten" for asthmatic bronchitis. The right eye showed microphthalmos, the anterior chamber was slightly shallow and the lens clear. On the posterior surface a grey membrane was seen, but no red reflex, the membrane being typical of fibroplasia. The left eye was of normal size, the anterior chamber showed nothing worthy of note and the lens was clear. Temporally on the posterior surface of the lens there was a thin grey wing-shaped membrane of the same appearance as the one on the right. Nothing special as regards the papilla. There were plenty of chorioiditic centres even in the macula. Right through the macula there extended a greyish fold of exudate, barely the width of the papilla, from below medially upwards temporally. The vessels were narrow. Nystagmus occasionally (Lindblad). The mother's toxoplasmosis test was positive, whereas the serological examination on the patient was negative. The intracutaneous toxoplasmin test on the patient was negative. W.R. negative. X-ray of skull negative. EEG normal. Nothing of note as regards the internal organs apart from asthmatic bronchitis. No hemangiomas. Mentally normal.

All the ophthalmic examinations were carried out by Dr. YNGVAR LINDBLAD, eye-specialist.

In order to obtain an idea of the incidence of fibroplasia the premature material has been investigated for the last few years

Table 1.

All premature infants treated between 1940 and 1949 at the Samariten Children's Hospital. They have been divided into different groups according to birth weights.

Birth weight g	Dead			Not examined			Examined			Total			Death per cent
	Boys	Girls	Total	Boys	Girls	Total	Boys	Girls	Total	Boys	Girls	Total	
< 1000	6	10	16	—	—	—	1	—	1	7	10	17	94
1001—1250	19	17	36	—	—	—	4	6	10	23	23	46	78
1251—1500	25	19	44	1	—	1	11	21	32	37	40	77	57
1501—2000	50	31	81	11	23	34	90	115	205	151	169	320	25
> 2000	6	2	8	1	1	2	21	20	41	28	23	51	16
Total	106	79	185	13	24	37	127	162	289	246	265	511	

at the Samariten Children's Hospital. This investigation was begun in 1948 by HEDLUND-KRISTIANSEN and LINDBLAD, and was intended to include all those infants under 1 500 g who were admitted between 1940 and 1947. It could not be completed, however, but nevertheless 31 were controlled. In the autumn of 1950 the investigation was resumed by LINDBLAD and the author, and it now includes all the premature infants admitted with a birth weight under 2 000 g during the decade 1940—49. Even twins of over 2 000 g have been included, if one of them has weighed less than this limit. Although they have not been admitted to the hospital, 10 of the former have been included for the sake of completion. All in all the investigation has included 511 children, 185 having died, 10 after leaving the hospital. The weight limits have been put at 1 000 g and under, 1 001—1 250, 1 251—1 500, 1 501—2 000 and over 2 000 g. The distribution will be seen from table 1.

No case of fibroplasia has occurred beyond the three already known. 289 infants or 85.6 per cent have been controlled, it having been impossible to examine 37 for various reasons. In all probability 23 of these 37 may be excluded from the possibility of fibroplasia on the strength of information obtained from parents

or relatives. If these 23 are included, 312 or 95,7 per cent have been controlled, while 14 or 4.3 per cent have not been examined. 3 have fibroplasia, making 1 per cent of surviving infants with a weight under 2 000 g at birth, and 7 per cent under 1 500 g.

Dr. HENNING MAGNUSSON has described 2 cases and called them the encephalo-ophthalmic syndrome. When these two cases were afterwards examined by Dr. LINDBLAD and the author, it was found that they belonged to the retrolental fibroplasia group, which conception Dr. MAGNUSSON also then supported.

Dr. OLOF OLSON, the eye-specialist at the Tomteboda Institute for the Blinds, and the author have examined all the 125 children (of school age) who have been admitted to this institute, and not one case has been discovered, though there was 1 suspect.

In order to ascertain whether any further cases of fibroplasia have been observed in Sweden, a circular has been sent to all the eye-specialists and pediatricians at clinics and general hospitals. It may be assumed that children with such ophthalmic changes as fibroplasia have been kept under control just at these hospitals. From the answers received as well as from information obtained in other ways, altogether 38 cases have been discovered, 20 of these being boys and 18 girls. The first two were born in 1945 and for those born between 1946 and 1950 the number of cases were 4, 7, 9, 9 and 7 respectively. The weight at birth varied between 920 and 2 200 g, 25 of the 38 being under 1 500 g (see table 2). The occurrence in Sweden will be seen from the accompanying map. This shows that the majority of cases are to be found in the most populous districts and particularly along the coasts.

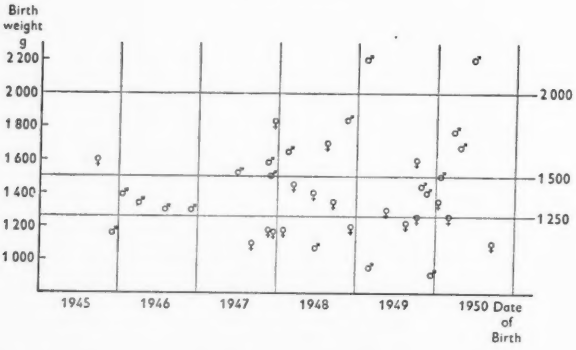
With the assistance of the Director of the Department of Child and Youth Psychiatry and Mental Deficiency a circular has been sent to all the asylums in the country in order to ascertain whether any cases of fibroplasia have been admitted under this or any other diagnosis. No fresh cases have been found.

It is possible that there are more cases of fibroplasia than are known, but they have not been diagnosed or have been misinterpreted. The disease has not been recognized here before.

At least in two cases toxoplasmosis occurred simultaneously

Table 2.

Birth weights and data of the 38 cases of retrolental fibroplasia in Sweden.



(all are not examined for this). Toxoplasmosis tests should therefore be taken on both mother and child, as well as W.R., since the luetic changes in the choroid may be easily mistaken for fibroplasia. Lues is, however, very rare in Sweden.

Children with retrolental fibroplasia may also have pathological changes in the brain which result in imbecility. Of the 38 cases in Sweden, 14 are mentally retarded, 11 appear to be mentally normal, and mental development is uncertain about the remainder. In 2 cases the ventriculograms have shown atrophy of the brain (Magnusson), though it was normal in a mentally normal girl (case 4). The electroencephalogram of this later patient was normal and also in case 6, though in 2 cases it was pathological (MAGNUSSON). As was to be expected, the electroretinogram (Karpe) showed no retinal function in eyes that had undergone considerable changes (case 1 and MAGNUSSON'S 2 cases).

The etiology is according to the opinion of the author probably infective and primarily of a virus type, though maybe not always uniform. Accordingly toxoplasmosis may sometimes possibly be a provocative element, or it may be simply a coincidence that it makes its appearance simultaneously. In cases of virus infection some sort of antibiotics, aureomycin, chloromycetin etc., should

A map of Sweden showing the distribution of the 38 cases of retrolental fibroplasia.

be tried at an early stage. Premature infants weighing below, say, 2 000 g at birth, should have their eyes controlled regularly during the first 6 months—1 year, so that possible treatment can be immediately started at the first sign of fibroplasia.

The Social Problems in connection with retrolental fibroplasia are of considerable significance. It is not always possible for these children to be kept at home till they reach school age and can be placed in blind institutes. The mothers are often unmarried and dependent on their work for their livelihood. Many of the children are unable to benefit from the teaching at blind institutes and must consequently be put under the care of asylums preferably as early as possible. As the incidence of retrolental fibroplasia increases, there is an increasing need of preparatory schools for mentally normal blind children. There are at present considerable difficulties in connection with the placing of those affected children who are mentally backward.

Summary

A report is given on the occurrence of retrolental fibroplasia in Sweden. Premature material has been examined from 1940—49 at the Samariten Children's Hospital in Stockholm, when infants weighing under 2 000 g at birth were examined by an eye-specialist. Of the surviving 326 children 95 per cent have been controlled, and of these 3 were found to be suffering from fibroplasia. There are 38 cases of this disease now known in Sweden, 20 boys and 18 girls, all born between 1945 and 1950. The weight at birth varies between 920 and 2 200 g, 25 being under 1 500 g. The etiology of the disease is supposed to be of infectious character, probably a neurotropic virus. Toxoplasmosis may be the cause in certain cases. Regular ophthalmic control is necessary during the first months in all premature infants weighing less than, say, 2 000 g. Finally the necessity for a special preparatory school to a blind institute has been emphasized for those who are mentally normal as well as institutes for the care of those who are mentally retarded.

Fibroplasie retrolentale en Suède.

Les observations de prématurés servant de base à ce travail proviennent de l'Hôpital d'enfants Samariten de Stockholm durant les années 1940—1949. Ces enfants de poids inférieur à 2 000 g ont été examinés par un spécialiste ophtalmologiste. Sur les 326 survivants 95 % furent soumis à un controle et parmi ceux-ci 3 seulement furent trouvés atteints de fibroplasie. Nous connaissons maintenant en Suède 38 cas de cette maladie: 20 garçons et 18 filles, tous nés entre 1945 et 1950. Le poids de naissance varie entre 920 et 2 220 g — 25 étant d'un poids inférieur à 1 500 g. L'étiologie de la maladie a un caractère infectieux provoqué probablement par un virus neurotrope atteignant la mère et produisant des lésions au niveau des yeux de l'enfant, avec possibilité de l'atteinte cérébrale — ceci étant en rapport avec l'état de prématurité. La toxoplasmose peut en être la cause dans certains cas. Si la nature infectieuse peut être retenue comme facteur étiologique, il est logique d'essayer les antibiotiques comme agents thérapeuthiques. Des exemples ophtalmologiques répétés sont donc nécessaires pendant les 1:ers mois de la vie chez tous les prématurés pesant moins de 2 000 g. Enfin il y a lieu d'envisager des écoles préparatoires — soit institutions pour aveugles destinées à ceux qui ont un niveau mental normal soit d'autres institutions spécialisés aux débiles.

Die retrolentale Fibroplasie in Schweden.

In den Jahren 1940—49 wurden im Samariten Kinderkrankenhaus in Stockholm vorzeitig Geborene, und zwar Säuglinge mit Gewicht unter 2 000 g, durch einen Facharzt für Augenheilkunde untersucht. 95 % der überlebenden 326 Kinder sind kontrolliert; 3 litten an Fibroplasie. Die Anzahl der in Schweden bekannten Krankheitsfälle von retrolentaler Fibroplasie beträgt 38, 20 Knaben und 18 Mädchen, alle geboren zwischen 1945 und 1950. Das Gewicht bei der Geburt schwankt zwischen 920—2 220 g, 25 wogen weniger als 1 500 g. Die Frequenz kann aus beigefügter Tabelle ersehen werden. Der Verfasser vermutet, dass die Ätiologie der Erkrankung von infektiöser Art sei, wahrscheinlich durch einen neurotropen Virus bedingt, welcher die Mutter befällt, Veränderungen in den Augen des Kindes und möglicherweise eine Schädigung des Gehirns hervorruft und welche eine Frühgeburt verursacht. In gewissen Fällen könnte Toxoplasmose die Ursache sein. Sollte es bewiesen werden, dass eine Virusinfektion die Krankheitsursache sei, so könnte man als therapeutisches Mittel Antibiotica anwenden. Daher ist regelmässige augenärztliche Kontrolle während der ersten Monate in allen Fällen von Frühgeburten angezeigt, in welchen der Säugling weniger als 2 000 g wiegt. Schliesslich wird sowohl die Ratsamkeit einer besonderen Vorbereitungsschule zur Blindenanstalt für die geistig Normalen als auch die Notwendigkeit der Anstaltspflege von Schwachsinnigen betont.

Fibroplasia retrolental en Suecia.

El material de estudio comprende prematuros examinados entre los años 1940—49 en el Hospital de niños Samariten de Estocolmo, se trataba de niños de peso por debajo de 2 000 g y que habían sido sometidos a exploración oftalmológica por especialista. De los 326 niños sobrevivientes un 95 % fueron controlados y de ellos en 3 se halló la presencia de una fibroplasia. Hasta ahora se conocen en Suecia 38 casos de esta enfermedad 20 niños y 18 niñas todos ellos nacidos entre 1945 y 1950. El peso en el momento de nacer variaba entre 920 y 2 200 g, 25 de los casos estaban por debajo de 1 500 g. El aumento de la frecuencia puede verse en el esquema acompañante. El autor presume que la etiología de la enfermedad pueda ser de caracter infeccioso, probablemente un virus neurotropo afectando a la madre y produciendo alteraciones oculares en el niño junto a posibles alteraciones cerebrales junto a un nacimiento prematuro. La toxoplasmosis pudiera ser la causa en ciertos casos. Siendo posible que la causa sea una infección por virus se han ensayado varios antibióticos como terapéutica. Los controles oftalmólogicos periódicos son necesarios durante los primeros meses en todos los prematuros cuyo peso sea inferior a 2 000 g. Finalmente la

necesidad de una escuela especial preparatoria para el Instituto de ciegos es recomendada para aquellos que tienen un nivel mental normal asi como tambien instituciones para el cuidado de los mentalmente retrasados.

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PROGRESS IN PEDIATRICS

Retrolental Fibroplasia

by

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A disease which has recently attracted increasing interest, particularly in the United States of America, is the so-called retrolental fibroplasia. It has been on the increase since 1937 and probably occurred before this, though only sporadically. The name was introduced in 1942 by TERRY of Boston. TERRY has reviewed the literature and, from 1873 to 1932, he found 50 cases showing changes in the eye, which, to a certain extent, resemble fibroplasia. REESE and PAYNE have also found similar abnormalities in the eye in some 20 cases reviewed between 1851 and 1938. KRAUSE has found 31 similar cases. The cases collected by REESE and PAYNE and KRAUSE are partly identical with those mentioned by TERRY. The changes have, however, mostly been unilateral and in full term infants, whereas in retrolental fibroplasia they are bilateral and premature infants are particularly affected.

The Occurrence of Retrolental Fibroplasia

So far retrolental fibroplasia has mainly been confined to the United States of America, though during the last few years occasional cases have been described in Europe. Thirteen cases have been published in France (FRANÇOIS, LELONG et al.), and one each in Spain (ARRUGA), England (WOLFF), and Denmark (HOLM). There are about 25 cases known in France (LELONG), 12 in Denmark (HOLM et al.), 7 in Finland (AF URSIN), and 2 in Norway (SALOMONSEN). In Sweden 1 case was demonstrated in 1948 at the Samariten Children's Hospital in Stockholm

(HEDLUND-KRISTIANSEN). A total of 38 cases has now been observed in Sweden in children born between 1945 and 1950, and these are discussed in a special report.

Types of Ophthalmic Diseases resembling Fibroplasia

According to REESE and BLODI 3 types can be identified. The first is the true retrolental fibroplasia found in premature infants, while the second is termed "persistent hyperplastic primary vitreous". This latter is a disturbance in the development of the eye; it is often unilateral and is usually to be found in full term babies with a functioning retina. It is congenital and appears between one day and about two weeks after birth. The eye is usually of normal size. The changes are sometimes slight, consisting only of a small hyaloid artery issuing from the papilla. The third type is termed "retinal dysplasia". It has nothing to do with premature birth, is bilateral and often associated with defects in the brain, heart, skeleton, polydactylism, cleft palate and other deformities.

The Development of Retrolental Fibroplasia

As has already been pointed out, retrolental fibroplasia in its true form occurs chiefly in premature infants. In his material REESE shows that out of 202 cases only 13 were full term babies. The development has been studied inter alia by OWENS and OWENS, GILGER and HEATH. HEATH divides the development into 3 stages. *The first* appears in the form of changes in the retina near the ora serrata and consists of dilated veins and tortuous arteries with hemorrhages (LAUPUS), edema and reduced transparency. There are often indications of these signs at birth and they always appear before the age of 7 months. The very earliest changes can only be seen microscopically. Outwardly there are no changes in the eye at this stage.

It is between the fourth and the sixteenth weeks that *the second stage* develops, when opaqueness and fibrillary formations make their appearance in the vitreous body, thus detaching the retina,

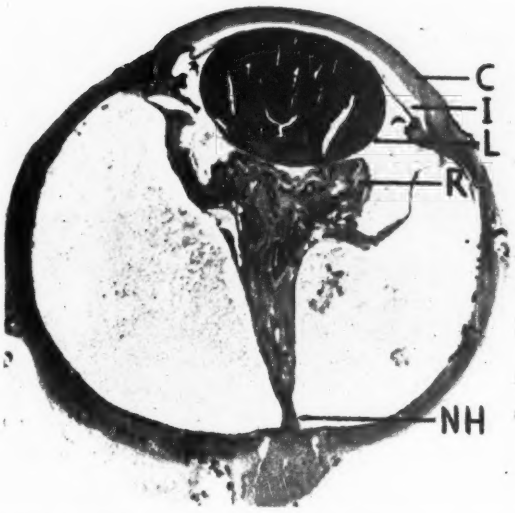

Fig. 1 (HEATH). Eye of infant born thirteen weeks prematurely with older stage of retinal vascular disease of prematurity affecting both eyes. Age at operation, 9 months; weight at birth, 3 pounds 7 ounces. *C* is infant cornea; *I*, infant iris; *L*, lens; *R*, retina, axial and balled behind the lens; *NH*, nerve head, dragged inward.

usually axially retrolentally. The process is now no longer reversible or even stationary, as it may be if the changes in the vitreous body are small. Sometimes a retinal fold can be lifted, and thus complete the final stage in the development. If the process continues, the lens will be pressed forward by the retina and the ciliary body which have been drawn into the changes by the connective tissue. Behind the lens a membrane forms, which, when light falls on it, produces a greyish white reflection with small vessels. The membrane presses the lens still further forward, thus making the anterior chamber more shallow and reducing the quantity of aqueous humor. The retina is often balled behind the lens. See figures taken from HEATH and WOLFF. The iris has usually a fetal blue or greyish blue colour. As a rule elongated ciliary processes can be seen in the periphery of the pupil if sufficiently dilated.

Fig. 2 (WOLFF). Retrolental fibroplasia. *A*, retina. *B*, capsule. *C*, membrane.

The third stage develops gradually between the ages of 1/2—2 years with degeneration and atrophy of the bulb. According to KING the disease is full developed by 4—6 months after birth. The eye lies sunken in its socket and the greyish white membrane can be seen in the pupil behind the lens. The membrane can push the lens forward so much that the cornea and the lens can coincide and the anterior chamber becomes entirely occluded. Very often there is nystagmus. Affected infants rub their eyes intensely, either because this gives a sensation of light or else it is relief from discomfort, just as in cases of inflammatory processes. Secondary complications may also arise, e. g. synechiae, glaucoma and cataract. The changes are bilateral, though the types may vary in both eyes. If the symptoms are slight on one side, the disease may appear to be unilateral.

The disease mentioned by KRAUSE and others does not always affect the eye alone, pathological tissue may also be found in the membranes and the ventricles of the brain, resulting in hydrocephalus or atrophy of the brain. Children with retrolental fibroplasia are often mentally retarded, 30—50 per cent, e. g. in KRAUSE'S group 37 out of 63. (Mental retardation occurs according to HESS et al. in about 10 per cent of all premature infants.) Before applying such a diagnosis, however, we must bear in mind the handicap these children have on account of their reduced sight or blindness.

Etiology

TERRY, GILGER, W. and E. OWENS, LEVINE, UNSWORTH et al. look upon retrolental fibroplasia as being postnatal, while KRAUSE, REESE and PAYNE, HOLM, KLIEN et al. consider it to be congenital. The occurrence of the disease varies considerably in different parts of the United States of America, ranging from nil to 30—35 per cent. The obvious increase can be seen from KRAUSE'S figures from 1945 to 1947 for infants whose weight at birth was under 1 500 g, the figures increasing from 11 to 45 per cent, and in 1948 to as high as 86 per cent. The fact that the percentage varies so much in different places must partly be attributed to what is included in "retrolental fibroplasia", whether slight reversible changes are taken into account and whether a birth weight limit has been selected. The increased incidence is also evident from the figures from the various blind institutes, where the disease often occurs in more than 50 per cent of the children under school age. In 1948, for example, 82 out of 148 (The Division of the Blind, Department of Education of Commonwealth of Massachusetts) and in the autumn of 1950 28 out of 56 freshly admitted blind children to Perkins Blind Institute, Massachusetts were found to be affected.

REESE and LEVINE et al. consider that the increase in retrolental fibroplasia is due to the fact that the number of small premature infants who survive now is greater than earlier. GILGER e. g. found in Cincinnati that there was an increase in 1943 from

29.1 per cent to 51.7 in 1947 of surviving children whose weight at birth was under 1814 g. HEATH and others do not consider this explanation satisfactory, HEATH pointing out that the disease has not increased everywhere, and in some places only a few cases have been noted, despite the increase in the number of surviving infants.

TERRY regards premature birth as being of importance, since the infant is thus deprived of certain important substances, hormones and antibodies.

The importance of mother's milk as a prophylactic against fibroplasia has also been taken into account. The frequency of fibroplasia is greater in The United States than anywhere else, and there mother's milk is less frequently used for feeding premature infants. Investigations carried out especially by EXLINE and HARRINGTON using cow's milk, showing no incidence of fibroplasia, must be considered proof of the fact that fibroplasia need not necessarily develop when artificial feeding is used for premature infants. GILGER is also of the same opinion.

REESE attaches great etiological significance to uterine hemorrhages during pregnancy, as he maintains that they occur in 30—60 per cent. HEATH and GILGER, on the other hand, do not believe that there is any such connection.

REESE, REESE and BLODI and KLIEN look upon angiomatous changes in the retina as being the primary cause of the disease. In these infants there seems to be a general tendency to hemangioma. They point out that hemangiomas of the skin are more frequent in this disease, 20 per cent according to TERRY and KRAUSE and 25 per cent according to REESE than is otherwise the case in premature infants, 3.2 per cent (HESS et al.). Others, however, do not attach much importance to hemangioma, and HOLM points out that the bilateral occurrence and the similar development do not support this.

The age of the mother, the number of children, one or several parturitions, the use of sedatives at childbirth, the manner of delivery, the Rhesus type, the use of sulfonamides or penicillin or an x-ray examination of the mother or child can be excluded as being the causes of the disease (EXLINE et al. and KINSEY et al.).

Earlier, the direct rays of light in incubators were looked upon as being an etiological factor, but after investigations carried out by HEPNER, KRAUSE and DAVIS, who found that fibroplasia develops in infants who have been kept in the dark the whole time, this theory can be excluded.

Great significance has been attached to the lack of various kinds of vitamins in the development of retrolental fibroplasia. WARKANY and SCHRAFFENBERGER found that a retrolental membrane developed in rats if the mother suffered from a lack of vitamin A. Nevertheless CLIFFORD and WELLER were able to prove that if vitamin A were dissolved in water and administered to premature infants, it did not prevent them from developing fibroplasia, whereas if dissolved in oil it seems more effective (KINSEY et al. and HEATH). GILGER, however, found no difference. The vitamin B complex, as well as vitamins C and D are not considered of any etiological importance, though such is not the case with vitamin E. It is particularly WILLIAM and ELLA OWENS who have studied the relation of this vitamin to the origin of the disease. The administration of vitamin A and a preparation of iron could produce a lack of vitamin E. For prophylactic purposes they have administered vitamin E in the form of alpha tocopheryl acetate, giving 150 mg. every day from the second to the eighth week to infants, whose weight was under 1 360 g at birth. Of 23 infants thus treated only 1 (4.4 per cent) developed fibroplasia, whereas in a control group of 78 cases 17 or 21.8 per cent were affected. The figures are small and cannot be altogether substantiated, HEATH maintaining that special circumstances may play a certain part. LEVINE, too, does not attach any great significance to the vitamin E prophylaxis. TERRY considers the lack of vitamin K, hypoprothrombinaemia, of importance for the origin of the disease. The excessive use of vitamins, particularly those soluble in water, is regarded as being injurious by HEPNER, KRAUSE and NARDIN.

UNSWORTH is of the opinion that anemia causes retrolental fibroplasia, while INGALLS attaches great importance to the lack of oxygen. According to GILGER the use of testosterone in cases of premature birth is of no etiological significance. EXLINE

and HARRINGTON lay etiological stress upon the initial decrease in weight, the administration of vitamins in high concentration, vitamins in aqueous dispersion and large amounts of iron. They have avoided such factors, and among 80 premature infants under 1 500 g at birth they have not had any cases of fibroplasia.

As HARLEY and MATHER have shown, congenital lues can cause changes in the choroid, resembling early fibroplasia.

Finally certain writers have dealt with the possibility of an infection to be found in the mother or infants as being the true cause of the disease (REESE, KRAUSE, WOLFF and HOLM). According to the opinion of the author it seems most likely an infection caused by a neurotropic virus in the mother appearing clinically manifest or occult thus producing the symptoms in the infant and even possibly causing premature birth. The recent increasing incidence during the last decade would support this theory.

Distribution between Boys and Girls

In most surveys boys predominate, e.g. TERRY and REESE present 96 boys out of 150 and KING 149 boys out of 238. In KINSEY'S and ZACHARIAS' group 14 per cent of the boys had retrolental fibroplasia but only 9.2 per cent of the girls (42 boys out of 301 and 34 girls out of 371).

Treatment

OWENS' experiments with the administration of vitamin E for prophylactic purposes seem to be promising in some measure. Other scientists, e.g. LAUPUS and BOUSQUET Jr. and LEVINE have not obtained any definite results with it. EXLINE and HARRINGTON have not administered any vitamin E at all and they have found no cases. Bearing in mind that the infants are deprived of essential substances from the mother owing to their premature birth, blood transfusions have been given to premature infants during the first few days after birth from mothers in the last third part of their pregnancy. So far it has not been

possible to draw any definite conclusions as to the value of this step (KINSEY).

Several workers (TERRY, OWENS, KRAUSE et al.) have tried roentgen therapy, but without effect.

ACTH and cortisone have been administered by LEVINE and REESE and BLODI and others with satisfactory results, while WOODS and others have not experienced any effects. REESE and BLODI gave 20 to 25 mg of ACTH daily for a period of fourteen days, possibly repeated. Thirteen babies with early retrolental fibroplasia have been treated in this manner with good results.

Certain promising results have been achieved by LELONG et al. with streptomycin locally in the eye. Should a virus infection prove to be the cause of the disease, early antibiotherapy should be resorted to.

The treatment of already developed fibroplasia is purely symptomatic. Should glaucoma or other troublesome complications arise, enucleation may be necessary. No other operative steps are of any value.

No definite treatment for retrolental fibroplasia has so far been discovered, nor do we know anything sure concerning its origin.

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CORRESPONDENCE

"Gargoylism, Forme Fruste"

To the Editor:

In the March issue of this journal SJØLIN (1951) reported a case of proportional dwarfism in a boy, 10 years of age, under the title of "Gargoylism, forme fruste". The syndrome was characterized by macrocephaly, open sutures and fontanelles, the anterior fontanel measuring 7 × 12 cm. The limbs were rather slender, but the fingers and toes were short, with deficient ossification in the distal phalanges. The sella turcica was comparatively small. The mental development was normal (the patient was, in fact, "the best at school").

By a process of elimination, the author, in his discussion of the case, arrived at a diagnosis of gargoylism. He tried to prove his conclusions by presupposing a forme fruste, but even such a condition should, of necessity, have some feature in common with the basic disease from which it has been named. Of this, there is no sign here. The reader searches in vain for any symptoms in the description of the case that might have confirmed the hypothesis of the author. There is no cardiac affection, no hernia, no liver or spleen enlargements, nor any clouding of the cornea. Instead of the kyphosis which is so typical of gargoylism, a lordosis is noted, being, in fact, described as "very marked". In gargoylism the sella turcica is ususally considerably elongated. Here, it is rather smaller than normal. Also, the unaffected mental condition is contrary to the usual findings in patients with gargoylism. Last but not least, to judge from the pictures of the patient, there is no indication in his appearance of the feature so characteristic of gargoylism, i.e., the gruesome face which is practically pathognomonic of this condition, and from which it has obtained its name.

The question may now be asked how the author has justified his diagnosis. All that remains is the dwarfed stature and the large head. As to the first, patients with gargoylism are *disproportionately developed* dwarfs with short neck, short and clumsy extremities, etc. The head is large but sutures and fontanelles do not long remain open.

As will be seen from this, the case under discussion is to such a degree "a forme fruste" as to possess no points of connection with gargoylism. Accordingly, it cannot be classified as such. The author might have found it worth his while to consider the caution displayed

by DE LANGE in labelling a case with various typical features of gargoylism, though without the kyphosis, the enlargement of the sella and the clouding of the cornea. For this reason, DE LANGE found it safer to apply an indifferent denotation alluding to the name of the patient (all the authors who have later dealt with this subject have, it may be added, accepted this particular case as a genuine case of gargoylism).

The demand for a proper title (see BORRIES) in this instance had been fulfilled if the author had choosen a heading such as "A case of proportional dwarfism", provided that no attention should be paid to the "pronounced universal osteosclerosis" ascertained by him. Since, in osteopoikilosis, gaping sutures and open fontanels are not uncommen even at an advanced age (BICHEL), this symptom also might have been included in the head-line.

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Gävle, Sweden, June 1st, 1951.

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To the Editor: **A reply to dr. Jelke.**

It ought to be evident from the case record published (Acta Paediaf trica 40: 165—174, 1951) that the differential diagnosis in the case ot dwarfism has been thoroughly discussed. We have not for a momen, considered this boy similar to the monstrous types of gargoylism. It is-however, to be expected, and case reports found in the literature confirm this, that there are many different types of gargoylism. The term "forme fruste" has, in fact, not been advanced by the author but is used by Henderson (1940) and others.

Dr. Jelke also mentions some symptoms of this patient which are not to be found in gargoylism. The fontanels and the sutures of the skull should not be open longer than normally. It may be remarked that Henderson (1940), Hurler (1919), and Kressler et al. describe delayed (several years) closure of the fontanels. Dr. Jelke says that lordosis is not seen in cases of gargoylism. It may be mentioned that this patient in spite of a straight spine clinically gives the impression of having a lordosis. This is caused by a spondylolisthesis. Engel (1940) states that lordosis may be seen in gargoylism.

The variability of symptoms in gargoylism is demonstrated by cases of Sheldon and Njaa where no dwarfism was found. Ruggles describes 3 siblings with gargoylism. Two had all the characteristic symptoms but the third did only show defects of ossification.

The symptoms of our patient which are consistent with the diagnosis of gargoylism are mentioned again here: Dwarfism, a large head with open fontanels and sutures, a face, if not monstrous, at least queer and ugly, short and plump fingers and toes with a rough and wrinkled skin, and defects of ossification. Finally two similar cases are occurring among the siblings of the patient.

Knud-Erik Sjølin.

BOOK REVIEW

HANS SELEY: *Annual Report on Stress.* Acta, Inc. Medical Publishers. Montreal, 1951. $ 10: —.

This volume represents the first of a planned series of annual supplements to the author's well known book "Stress; The Physiology and Pathology of Stress". This annual report is not meant solely as an abstract review of articles on the general adaptation syndrome but is a critical correlation of pertinent facts. Honestly, the author confesses that he cannot do such an evaluation quite impersonally. The book is subdivided into two main sections: The General Physiology and Pathology of Stress and The Special Physiology and Pathology of Stress. The second section is of special interest to the clinician. It contains data on the action of stress upon the various targets (individual organs, metabolic changes and on certain functional responses). The organization of the material makes it easy to find a wanted information and everything is made to facilitate the reading of the book and the practical use of it. No less than 3000 articles published during the last academic year have been quoted and evaluated. A first review on recent publications on the general adaptation syndrome was written 1950 in which 5500 articles that had appeared since 1937 were abstracted. The literature dealing with this subject is thus tremendous and it is not possible for one single physician or laboratory man to survey everything that is written. To those who want to follow the development of the conception of the general adaptation syndrome this volume is inestimable.

The reviewer will agree with the editorial remarks in The Lancet: the works of Seley must be read by anyone who seriously professes to follow the most important trend of medicine at the opening of the second half of the twenteeth century.